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1995

ANNUAL REPORT
NATIONAL INSTITUTE OF DENTAL RESEARCH
FY 1995
(OCT. 1, 1994 - SEPT. 30, 1995)



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00497-07 OD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Forecasting Dental Health and Utilization Using A Microsimulation Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson Director, OD DEODP NIDR
Zion, Gary R. Computer Programmer, HAS ASHAB DEODP
Oldakowski, Richard J. Chief, SPU ASHAB DEODP

COOPERATING UNITS (if any)

Cornell University, Department of Sociology, Ithica, New York and the University of Michigan, Ann Arbor, MI

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIH NIDR, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.44

PROFESSIONAL:

.44

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A computer model which will generate condition forecasts of future tooth loss, dental status, service utilization and expenditures for individuals and families in the US was developed under a contract with Cornell University. The forecasts will be developed in considerable sociodemographic detail. Several noted dental specialists and modeling experts were also consultants to the project. Microsimulation is the approach being used. The development of the model and the production of initial forecasts has been completed. Tests of the full model are being conducted by DEODP staff. The model has been adapted to NIH computer systems and resides on a RISC where new SAS software to analyze the output is being developed. Starting from a representative sample of persons and families, the NIDR micro model will forecast tooth loss, dental health conditions, and dental service use for persons identified by age, gender, race, education, income, and other putatively important explanatory variables. Policy experiments with the full model are planned both for past times and also for future times. As a framework for synthesizing research findings, the NIDR micro model will provide a vehicle for carrying out experiments in which the latest dental research can be applied consistently and systemically to key dental policy issues.

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1995

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00583-03 OD

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The NIDR Amalgam Study and Health Effects Study Protocol

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, DEODP
Albertini, T. Special Assistant to Director, DEDOP
Brown, L. J. Director, DEODP

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.46

PROFESSIONAL:

0.46

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is a major study investigation whether there is any evidence of adverse health effects attributable to exposure to dental amalgams in a specific adult populations. The NIDR amalgam Study involves 1166 Air Force Veterans, roughly representing a 50% subsample of the Air Force Health Study participants. These participants were examined for scores of medical conditions, including many which would potentially be affected by exposure to mercury. Dental Examinations and blood and urine samples were obtained from the 1166 participants who are in the NIDR Amalgam Study. The exact type of restorative material used in exposure was defined as the total number of surfaces having an amalgam restoration present. All soft tissue conditions detected were obtained. Mercury levels in blood and urine were recorded in ug/l.

The amalgam exposure for this cohort was determined to be rather high, averaging 20 surfaces on average. The blood and urinary Hg levels were found to be low, averaging 2.9 ug/l and 3.1 ug/l, than 15 ug/l, but these levels could not be ascribed to dental amalgam exposure. There was a clear dose response association found between amalgam exposure and blood Hg levels.

The health data will be obtained from the AFHS scientists shortly and the tedious, complex analytical process begun.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00621-02 OD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Distribution of Dental Restorative Materials in a military population

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Albertini, Tullio F.	Special Asst. for Program Mngt, OD DEODP NIDR
Kingman, Albert	NIDR Chief Statistician, OD DEODP NIDR
Brown, L. Jackson	Director, OD DEODP NIDR

COOPERATING UNITS (if any)

United States Air Force, AL-AEOP

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland

TOTAL STAFF YEARS:

.50

PROFESSIONAL:

.45

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An important aspect of the NIDR Amalgam Study of Air Force Veterans is an inventory of dental restorative materials found in the study population which can serve as the basis for constructing exposure variables. This project reports on the distributions of dental materials found in a military population. Dental examinations were performed on 1166 male veterans to obtain tooth and surface specific data on existing restorations. Dental materials were classified into five categories: amalgams, resins, porcelains (including cements and temporaries), gold and other metals. The mean age of these veterans was 52.8 years. Overall, 5.2% of the participants were edentulous. Dentate individuals averaged 19.9 amalgam surfaces per person, 18.4 resin and porcelain surfaces combined and 10.4 surfaces for gold and other metals combined. Slightly more than 1/2 of all restored surfaces in dentate individuals were restored with amalgam. The mean number of tooth surfaces restored with PCT, Gold and Other Metals increased with age while amalgams and resins showed no increase after adjustment for number of teeth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00634-02 OD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Retrospective Analysis of Dental Manpower Supply Projections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Albertini, Tullio F. Special Asst. for Program Mngt, OD DEODP NIDR

COOPERATING UNITS (if any)

Bureau of Health Profession, HRSA, PHS	Clark, Norman	BHPR, HRSA
Bronstein, Gloria	BHPR, HRSA	Bernstein, Stuart
Herbert Traxler	BHPR, HRSA	BHPR, HRSA

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland

TOTAL STAFF YEARS:

.15

PROFESSIONAL:

.15

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study consists of a retrospective analysis of the accuracy of dentists supply projections contained in eight reports to the President and Congress on the supply of health professional in the U.S. This study represents the first in-depth external evaluation of dentists federal supply projections. Since the reports are important sources of information for dental educational institutions, it is important to provide planners and researchers with an assessment of the accuracy of such federal estimates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00639-02 OD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NHANES III - Workgroup Participation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brown, L. Jackson

Director, OD DEODP NIDR

Kingman, Albert

NIDR Chief Statistician, OD DEODP NIDR

Albertini, Tullio

Special Asst. for Program Management, OD DEODP NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIH NIDR, Bethesda, Maryland

TOTAL STAFF YEARS:

.40

PROFESSIONAL:

.35

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past Fiscal Year, OD staff have provided management oversight, professional advice and participated in the analysis of the oral examination data from the first phase of the National Health and Nutrition Examination Survey-III. This survey is a major source of national data on the prevalence of oral health conditions in the US population. The staff participated in the development of analytical and publication workplans including periodontal disease, caries, tooth conditions and restorative treatment needs, and a number of other specific topical areas. Participation also included the preparation and review of abstracts, review of preliminary data runs, evaluation of data quality, development of summary measurements, analysis of tabulations, development of publication schedules and outlines, and the preparation of presentations and papers for dissemination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00640-02 OD

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Associations Between Amalgam Exposure and Hg Levels in Urine and Blood

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, OD DEODP
Albertini, T. Special Asst. to Director, OD DEODP
Brown, J. Director, OD DEODP

COOPERATING UNITS (if any)

USAF AL/AEOP
Brooks Air Force Base, TX

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.15

PROFESSIONAL:

0.15

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dental Examinations and blood and urine samples were obtained from a sample of 1166 adult Air Force Personnel participating in the AFHS. The exact type of restorative material used in dental fillings was recorded for each affected surface. Amalgam exposure was defined as the total number of surfaces having an amalgam restoration present. Mercury levels in blood and urine were recorded in ug/l.

The amalgam exposure for this cohort was determined to be rather high, averaging 20 surfaces on average. The blood and urinary Hg levels were found to be low, averaging 2.9 ug/l and 3.1 ug/l for total mercury concentrations, in whole blood and urine, respectively. There were 11 participants who had Hg levels greater than 15 ug/l, but these levels could not be ascribed to dental amalgam exposure. There was a clear dose response association detected between amalgam exposure and urinary Hg levels, and a statistically significant, but none meaningful, dose response found between amalgam exposure and inorganic blood Hg levels. No association was detected for total Hg in blood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00641-02 OD

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Direct Versus Indirect Estimation of Amalgam Exposure in Adult Males

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, DEODP
Albertini, T. Special Asst. to Director, DEODP
Brown, J. Director, DEODP

COOPERATING UNITS (if any)

USAF AL/AEOP
Brooks Air Force Base, TX

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.03

PROFESSIONAL:

0.03

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Investigators have suggested that the effects of exposure to mercury vapor from dental amalgams be investigated by using data bases including various health outcomes measures and coronal caries DFS scores. In such data bases one is confronted with having to estimate amalgam exposure by an indirect method (for example, assume that all restorations on occlusal surfaces are amalgam). The effect of misclassification was investigated by using the NIDR Amalgam Study data base. In this study the coronal caries scores were obtained by the standard NIDR criteria used in epidemiological surveys. All restorative materials found in these study participants were also documented at the surface level. Thus, direct estimation of the total number of amalgam surfaces for each participant was possible as well.

The effect of various indirect estimates of amalgam exposure were estimated. The result of assuming that all occlusal restorations involved amalgam material produced positively biased estimates of amalgam exposure which ranged from 30% to 70% of their true values. The magnitude of the bias was not consistent over the age range 40 - 70 years of age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00652-01 OD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevalence of soft tissue lesions in a military population

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Albertini, Tullio F.	Special Asst. for Program Mngt, OD DEODP NIDR
Kingman, Albert	NIDR Chief Statistician, OD DEODP NIDR
Brown, L. Jackson	Director, OD DEODP NIDR
Kleinman, Dushanka	Deputy Director, OD NIDR

COOPERATING UNITS (if any)

United States Air Force, AL-AEOP

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland

TOTAL STAFF YEARS:

.15

PROFESSIONAL:

.15

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

An important aspect of the NIDR Amalgam Study of Air Force Veterans is an examination of oral soft tissue lesions which may be associated with amalgam restorations. Previous investigators have reported associations of amalgam tattoo and some lichenoid lesions with dental amalgam restorations. This project reports on the distributions and characteristics of soft tissue lesions found in a military population and examines the relationship of these lesions to restorative materials found in this study population. Oral examinations were performed on 1166 male veterans to obtain data on pigmented and white lesions. Additional information gathered included a description of the surface morphology and color of the lesions as well as the anatomical location of the lesions in the oral cavity. A complete inventory to existing dental restorations including identification of dental restorative material was also performed. The mean age of these veterans was 52.9 years. Overall, 5.2% of the participants were edentulous. Preliminary analysis indicates that 149 individuals (12.8%) had at least one soft tissue lesion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00655-01 OD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Natural History of Periodontal Diseases Using a Computer-Assisted Digital Image

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Albandar, Jasim M. Visiting Scientist, DEODP
Brown, L. Jackson Director, DEODP OD NIDR
Zion, Gary R. Computer Programmer, HAS ASHAB

COOPERATING UNITS (if any)

Dental Faculty, University of Oslo, Norway

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIH NIDR, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.27

PROFESSIONAL:

.27

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A computer program, based on the X11 window system operating in a UNIX environment, is developed for processing digital images of dental radiographs. It utilizes algorithms for warping and subtracting serial digital images by the use of reference points identified on the two images, and contains features for correction of film contrast differences. Regions of interest which include the interproximal areas of the teeth are defined with the cursor and analyzed by the program. This program is useful in the study of the change in the alveolar bone density and attachment level of teeth, and may provide a valuable tool in the study of the natural history of periodontal diseases, and the study of bone porosity.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00656-01 OD

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Progression and Risk Markers of Early Onset Periodontitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

Albandar, Jasim M.

Visiting Scientist, DEODP NIDR

Brown, L. Jackson

Director, OD DEODP NIDR

Brunelle, Janet A.

Staff Scientist, ASHAB DEODP NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIH NIDR, Bethesda, Maryland

TOTAL STAFF YEARS:

.20

PROFESSIONAL:

.20

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Early onset periodontitis is a disease characterized by a rapid progression of loss of the tooth supporting tissues in adolescents and young adults. This study was undertaken to study the pattern of progression of this disease and to identify potential risk factors. Adolescents with early onset periodontitis were identified within a population of 14,000 pupils in grades 8 to 12 examined by a national survey of the oral health of US children conducted by NIDR during the 1986/87 school year. A group of controls were matched to disease cases on gender, race, age, geographic location and metropolitan status. The cases and controls were followed over a period of 6 years during which clinical measurements were undertaken to monitor the attachment loss, caries, dental fillings, gingival inflammation and local plaque retaining factors. Subgingival plaque and blood samples were collected from these subjects, and microbiological and immunological assays were performed on the samples. The analyses are underway to assess the potential of these factors in discriminating between the cases and the controls.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00657-01 OD

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Composite Summary Measures and Global Test Statistics in Multiple Outcomes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kingman, A. Chief Statistician, DEODP

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director, DEODP

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.10

PROFESSIONAL:

0.10

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical studies in which multiple outcomes are evaluated pose methodological dilemmas. One may test for group differences for each outcome measure separately, and then adjust the α level with a Bonferonni type correction, or attempt to combine these assessments in a variety of ways to maintain the experimentwise type I error at size α .

Two general approaches for combining outcome assessments were considered: composite outcome measures (outcomes within an individual were summarized and the summary measure used in the analysis); and global test statistics (combining the individual test statistics into one global test). Global test statistics are linear combinations of the dependent individual test statistics (GLS was used here). Dichotomous outcome responses in models with several covariates were considered. Quasilikelihood methods were used to estimate correlations between individual test statistics. Simulations are being conducted to compare the global test procedures with the composite outcome measure method.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00464-08 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Longitudinal Study of Oral Manifestations of HIV-Infection in a Military Population

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.	Chief, ASHAB	DEODP, NIDR
Nowjack-Raymer, Ruth	Public Health Res. Spec., DPHPB	DEODP, NIDR
Brunelle, Janet A.	Statistician, ASHAB	DEODP, NIDR
Kaste, Linda M.	Senior Staff Fellow, ASHAB	DEODP, NIDR
Kleinman, Dushanka V.	Deputy Director,	OD, NIDR
Stack, Kathleen	Public Health Intern, HPDPB	DEODP, NIDR
Oldakowski, Richard J.	Chief, SPU, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

Walter Reed Army Institute of Research

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Office of the Branch Chief

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.47

PROFESSIONAL:

0.57

OTHER:

1.90

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

United States military personnel and dependents who have tested seropositive for the Human Immunodeficiency Virus (HIV) are given medical examinations and treatment at Walter Reed Army Medical Center. Subjects are also invited to participate in a research protocol to study the natural history of HIV infection, conducted by the Walter Reed Army Institute of Research. An oral health research component conducted by NIDR is a part of this natural history study. Subjects were enrolled in the oral health component from 1989 to 1994 and returned approximately every six months for re-evaluation of their oral health status and for other tests and procedures.

The oral component includes examinations by an oral medicine specialist for soft tissue lesions, periodontal conditions (gingival bleeding, plaque and calculus indices, presence of inflammation/destruction of interdental papillae, and presence of erythematous banding), and caries, and the acquisition of plaque, saliva, and mucosal smears for tests for candida. A questionnaire obtains information from subjects on demographic characteristics, health care utilization, oral symptoms, oral hygiene practices, and tobacco and alcohol intake. Information is obtained from the main Walter Reed Army Institute study on other factors such as Walter Red staging, CD4+ counts, and medications. The purpose of the study is to document the prevalence and incidence of oral pathologic conditions in relation to the stage of HIV infection and systemic disease. Risk factors associated with these conditions are also characterized, and the role of oral manifestations as early predictors or markers of disease progression are studied. Areas of emphasis are mucosal diseases, periodontal conditions, candida infections, and salivary constituents. Field data collection for this study ended in August 1994. Approximately 814 persons were seen at least once. About 40% of these persons have had 3 or more exams and 13% had at least 5 exams.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00496-07 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determinants of Permanent Tooth Loss in Connecticut and North Carolina

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marcus, Stephen E	Senior Epidemiologist, ASHAB	DEODP, NIDR
Brown, L. Jackson	Director	DEODP, NIDR
Winn, Deborah M	Chief, ASHAB	DEODP, NIDR
White, B. Alexander	Senior Dental Res. Investigator, ASHAB	DEODP, NIDR
Brunelle, Janet A.	Statistician (Health), ASHAB	DEODP, NIDR
Redford, Maryann	Public Health Specialist, HPDPB	DEODP, NIDR

COOPERATING UNITS (if any)

University of Connecticut, Farmington, Connecticut
University of North Carolina, Chapel Hill, North Carolina

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.42

PROFESSIONAL:

0.37

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this study is to measure permanent tooth loss and the factors which influence it. The study was conducted in two phases. The first phase is independent of the second and is a complete study without the second phase. Specific aims of Phase I are to describe the: (1) biological condition of extracted teeth, (2) sociodemographic, attitudinal, economic, and care-seeking characteristics of individuals who have extractions, and (3) selected characteristics of the dental providers who perform the extractions. Phase II was conducted after the first phase and collected information on patients whose teeth were treated with dental services that are alternatives to extraction for given biological conditions. These teeth will be controls for the extracted teeth and will allow the estimation of a model which explains the factors which influence the choice between extraction and its alternatives. The same dental practices were used for both phases. Data from both Phases will be used to develop a more complete explanation of the relative significance of a multiplicity of factors for tooth loss.

Phase I of the project has been completed and data collection for Phase II is nearing completion. Some aspects of the Phase II protocol were redesigned based on Phase I experience. Chief among the modifications: a new set of extraction patients were enrolled into the study, eligibility criteria were based on the treatments patients received, revised data collection forms (and the addition of a new log form to collect information about all patients seen by the participating dentist), two new procedures manuals, revised sampling strategy for dentists and patients, and a revised number of patients each dentist was expected to enroll into the study. The new protocol is more complex than that used to guide Phase I or intended for Phase II; increased training and monitoring/support from the program office in each site, however, has ensured high quality data collection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00527-05 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Software for Analyzing Data From Complex Dental Surveys

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.

Chief, ASHAB

DEODP, NIDR

COOPERATING UNITS (if any)

National Center for Health Statistics, Center for Disease Control & Prevention
Hyattsville, Maryland

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.11

PROFESSIONAL:

0.01

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The development of data analysis techniques that are appropriate for the analysis of complex sample surveys is an ongoing project. Several software packages such as SUDAAN and Westvar are available for the analysis of complex sample survey data. They use different mathematical algorithms (e.g., Taylor series vs "Jackknife" procedures) and differ in other features as well. Data from multiple large national oral health surveys are being used to analytically test and evaluate these software packages in five areas:

- (1) appropriate methodology for complex survey samples
- (2) portability
- (3) reliability/numerical accuracy
- (4) computational efficiency
- (5) ease of use
- (6) handling of very large sample sizes

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00544-05 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Periodontal Health in Adolescent Americans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brunelle, Janet A.	Statistician (Health), ASHAB	DEODP, NIDR
Brown, L. Jackson	Director	DEODP, NIDR
Albandar, Jasim	Visiting Scientist	DEODP, NIDR

COOPERATING UNITS (if any)

Westat, Inc.; SUNY, Buffalo; University of Minnesota; University of Tennessee; Columbia University; VPI; Medical College of Virginia

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.79

PROFESSIONAL:

0.79

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A major study to relocate, re-examine and collect risk factor information on children who were classified as having localized juvenile periodontitis, generalized juvenile periodontitis or incidental loss of periodontal attachment in the 1986-87 National Survey of the Oral Health of U.S. Schoolchildren. Research objectives are to: (1) assess the progression of periodontal destruction among the cases of early onset periodontitis, (2) characterize the microbial ecology of the sub-gingival plaque among persons with early onset periodontitis, and (3) compare the presence and concentration of selected putative pathogens and high-resistance factors among individuals with early onset periodontitis to controls.

A sample of subjects were selected whose oral examinations from the 1986-87 National Survey of the Oral Health of U.S. Schoolchildren indicated early onset periodontitis or other severe periodontal problems. From the same national survey two controls per case matched by age, gender, race and geographic location were also located and invited to participate in the study. During FY'93, examinations were made on approximately 265 young people 19-25 years of age. Full mouth oral examinations for periodontal measures and dental caries were conducted. Biological specimens, blood, gingival crevicular fluid and subgingival plaque fluid were also collected. A questionnaire including such variables as medical history, family history, and dental utilization was administered at the time of the oral exam.

Dental examination data was edited and summary measures derived. Laboratory analyses of serum, plaque and gingival crevicular fluid were conducted by a number of different laboratories. Relationships among the many clinical and laboratory variables measured are being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00567-04 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Physiology of Aging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Streckfus, Charles F.	Senior Staff Fellow, ASHAB	DEODP, NIDR
Adesanya, Margo R.	Dental Officer, ASHAB	DEODP, NIDR
Brown, L. Jackson	Director	DEODP, NIDR
Furman, Lawrence J.	Dental Epidemiologist, ASHAB	DEODP, NIDR
Brunelle, Janet A.	Statistician (Health), ASHAB	DEODP, NIDR
Oldakowski, Richard J.	Chief, SPU, ASHAB	DEODP, NIDR
Kingman, Albert	Senior Statistician, OD	DEODP, NIDR
Winn, Deborah M.	Chief, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

National Institute on Aging and
Johns Hopkins Bayview Medical Center

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.88

PROFESSIONAL:

1.28

OTHER:

0.60

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The oral research component of the Baltimore Longitudinal Study of Aging (BLSA), since its inception in 1978, has been designed and conducted by the NIDR to evaluate the physiological and pathological factors that influence the oral health and function of individuals of different ages. The BLSA is a large longitudinal study with an open-panel design conducted by the National Institute on Aging to investigate a range of scientific hypotheses about the aging process. Human volunteers return periodically to the BLSA site to undergo multiple health assessments including the oral health evaluation made by Branch staff. The Division of Epidemiology and Oral Disease Prevention has broadened the scope of the oral research component to include studies of alveolar bone loss in the oral cavity, the detection and application of oral molecular biological markers for systemic disease, and an expanded periodontal evaluation implementing protein markers and DNA microbial probes for early disease detection. The oral epidemiology component is also working with the BLSA to increase minority enrollment thereby increasing the diversity of the study's population base. The implementation of these additional areas of investigation within the BLSA, present an opportunity to enhance the overall understanding of age related changes in the oral cavity.

05

Date	Time	Location	Activity	Remarks
05/01/2020	08:00	Site A	Inspection	Normal
05/01/2020	09:00	Site B	Inspection	Normal
05/01/2020	10:00	Site C	Inspection	Normal
05/01/2020	11:00	Site D	Inspection	Normal
05/01/2020	12:00	Site E	Inspection	Normal
05/01/2020	13:00	Site F	Inspection	Normal
05/01/2020	14:00	Site G	Inspection	Normal
05/01/2020	15:00	Site H	Inspection	Normal
05/01/2020	16:00	Site I	Inspection	Normal
05/01/2020	17:00	Site J	Inspection	Normal
05/01/2020	18:00	Site K	Inspection	Normal
05/01/2020	19:00	Site L	Inspection	Normal
05/01/2020	20:00	Site M	Inspection	Normal
05/01/2020	21:00	Site N	Inspection	Normal
05/01/2020	22:00	Site O	Inspection	Normal
05/01/2020	23:00	Site P	Inspection	Normal
05/01/2020	00:00	Site Q	Inspection	Normal
05/01/2020	01:00	Site R	Inspection	Normal
05/01/2020	02:00	Site S	Inspection	Normal
05/01/2020	03:00	Site T	Inspection	Normal
05/01/2020	04:00	Site U	Inspection	Normal
05/01/2020	05:00	Site V	Inspection	Normal
05/01/2020	06:00	Site W	Inspection	Normal
05/01/2020	07:00	Site X	Inspection	Normal
05/01/2020	08:00	Site Y	Inspection	Normal
05/01/2020	09:00	Site Z	Inspection	Normal
05/01/2020	10:00	Site AA	Inspection	Normal
05/01/2020	11:00	Site AB	Inspection	Normal
05/01/2020	12:00	Site AC	Inspection	Normal

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00572-03 ASHA

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cost and Utilization of Dental Services - Second National Medical Expenditure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

White, Benjamin A.	Senior Dental Research Investigator, ASHAB	DEODP, NIDR
Kaste, Linda M.	Senior Staff Fellow, ASHAB	DEODP, NIDR
Marcus, Stephen E.	Senior Epidemiologist, ASHAB	DEODP, NIDR
Oldakowski, Richard J.	Chief, SPU, ASHAB	DEODP, NIDR
Zion, Gary R.	Computer Programmer, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.47

PROFESSIONAL:

0.47

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In 1987, the National Medical Expenditure Survey (NMES) II was conducted by the Center for General Health Services Intramural Research, Agency for Health Care Policy and Research. The survey provides extensive information on health expenditures by or on behalf of American families and individuals, the financing of these expenditures, and each person's use of services during the period from January 1 to December 31, 1987. The NMES II Household Survey is based on a national probability sample of the civilian, noninstitutionalized population living in the community. The sample is designed to provide a larger representation of population groups of special policy interest to the Federal Government than would have been obtained from a random sample. These groups include poor and low income families, the elderly, the functionally impaired, and black and Hispanic minorities.

As part of NMES II, information was collected on the type of dental services provided, total dental expense, and sources of payment for all dental services. This project uses these data to examine several issues related to dental services, such as: (1) overall dental utilization and expenditure patterns; (2) geographic variation in practice patterns for dental services; (3) demographic and socioeconomic variation in use of dental services; (4) associations between the use of dental services and other health care services; (5) relationship between the use of dental services and reported oral and general health status; (6) use of dental services by Native Americans and Alaska Natives; (7) use of dental services and health related behaviors, including care seeking and preventive care; (8) usual source of medical and dental care and reasons for lack of a usual source of dental care; (9) health insurance status and use of dental services; and (10) dental visits for accidents and injuries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00573-03 ASHA

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Patterns of Care, Outcomes, and Cost of Oral Cavity and Pharyngeal Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

White, B. Alexander	Senior Dental Research Investigator, ASHAB	DEODP, NIDR
Winn, Deborah M.	Chief, ASHAB	DEODP, NIDR
Marcus, Stephen E.	Senior Epidemiologist, ASHAB	DEODP, NIDR
Kleinman, Dushanka V.	Deputy Director	OD, NIDR
Garcia, A. Isabel	Senior Policy Analyst	OD, NIDR

COOPERATING UNITS (if any)

Applied Research Branch, Surveillance Program, Division of Cancer Prevention and Control, National Cancer Institute and Division of Beneficiary Studies, Office of Research, Health Care Finance Administration

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.33

PROFESSIONAL:

0.28

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The SEER/Medicare Linkage Project is a collaborative effort by the NCI and the Health Care Financing Administration (HCFA) to link the NCI's Surveillance, Epidemiology, and End Results (SEER) Program data base, which contains information on cancer cases diagnosed and reported in nine geographically distinct population-based tumor registries, and HCFA's Medicare statistical system (MSS), which contains extensive billing information for the health care of the disabled and more than 95 percent of the elderly. The DEODP is working in collaboration with the National Cancer Institute (NCI) to analyze the data on oral and pharyngeal cancer patients. This extremely large and complex data base will allow for extensive analysis of patterns of health care among persons 65 years of age and older subsequent to a diagnosis of oral or pharyngeal cancer.

Specific objectives of this study are to: (1) identify regional (SEER) variations in the choice of first-course of cancer directed therapy among individuals 65 years of age and over with oral cavity and pharyngeal cancer, by site of cancer; (2) add to the information currently available concerning the effectiveness of alternative therapies used to manage oral cavity and pharyngeal cancer among the elderly population; and (3) estimate the lifetime costs associated with oral cavity and pharyngeal cancer. For the years 1984 to 1989, 7,014 incident cases of oral cavity and pharyngeal cancer are contained in the data base, representing over 32,400 person-years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00579-03 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dental Caries and Selected Microbiological Determinations in Hispanic Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brunelle, Janet A. Statistician (Health), ASHAB DEODP, NIDR

COOPERATING UNITS (if any)

Stanley B. Heifetz University of Southern California
Jorgen Slots University of Southern California

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.02

PROFESSIONAL:

0.02

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

As part of a sealant program being conducted in inner city schools in Los Angeles, approximately 190 Hispanic children in grades 2 and 3 (7 and 8 years of age) were selected for inclusion in a study of caries and microbiological correlates. One clinical examiner scored the presence of decayed, missing or filled deciduous (dmfs) and permanent (DMFS) teeth. Before the clinical exam, children rinsed with 5 ml sterile distilled water for 15 seconds to provide microbiological samples. Questionnaires to check residency and heritage information were also obtained.

All samples were delivered to the laboratory on same day and plated on TYCSB agar for mutans streptococci and Rogosa SL agar for Lactobacillus. Total viable counts (CFU/ml of salivary rinse) of S. mutans, S. sobrinus and Lactobacillus species were determined for each child. Analyses of the associations between microbiological counts of S. mutans, S. sobrinus and Lactobacillus and deciduous and permanent caries status were made. For deciduous caries, distributions of children by presence or absence of caries and 'none' or 'any' bacterial counts were made. X² tests of these distributions indicated strong associations between dmfs and both mutans streptococci and Lactobacillus (p < .001). Mean dmfs scores (standard errors) for 'none' and 'any' mutans streptococci were 1.61 (0.6) and 8.05 (0.6) respectively and 3.88 (0.8) and 9.36 (0.7) for Lactobacillus, respectively. Mean caries levels increased as counts of mutans streptococci or Lactobacillus increased.

These data reaffirm the strong association between dental caries in the deciduous dentition and mutans streptococci and lactobacillus. Further analysis of bacterial counts, dental caries and acculturation are being made.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00580-03 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Occupation and Reproductive Health of Women Dentists

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kaste, Linda M.	Senior Staff Fellow, ASHAB	DEODP, NIDR
Doan, Linh A.	Computer Programmer, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

American Dental Association, University of North Carolina, and NIEHS

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.50

PROFESSIONAL:

0.45

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A national study assessing the relationship between occupational exposures related to the practice of dentistry and the reproductive health of women dentists was conducted. Among the occupational exposures of interest are mercury amalgam and nitrous oxide. The target study sample included approximately 5,500 women who graduated from dental school from 1977 to 1986 and were 31-40 years old in the spring of 1992. The sample was chosen to allow for the women to have had an opportunity to have dental occupational exposures during reproductive ages. Questionnaires were mailed to the women inquiring about occupational practices and reproductive history. The methodology builds upon work conducted at NIEHS on occupational exposures focusing on amalgams and nitrous oxide and reproductive outcomes, including time to pregnancy and spontaneous abortion. This is the first time a self administered questionnaire was used to collect this type of data from dentists. An initial mailing, post-card follow-up and thank you, second questionnaire, and a follow-up letter from the American Dental Association, for a total of four mail contacts, were made during fiscal year 1993. An additional mailing via registered mail occurred in FY 1994. A response rate near 70 percent has been achieved. Data entry was completed and data editing, and analysis continue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00581-03 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biomarkers for Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.	Chief, ASHAB	DEODP, NIDR
Schwartz, Joel L.	MEDIB	DEODP, NIDR
Diehl, Scott R.	Chief, MEDIB	DEODP, NIDR
Robbins, Keith	Chief, LCDO	DIR, NIDR
Yeudall, Andrew	Visiting Associate, LCDO	DIR, NIDR
Cardinali, Massimo	Visiting Associate, LCDO	DIR, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.30

PROFESSIONAL:

0.25

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objective of this study is to evaluate the recurrence of white and/or red oral soft tissue lesions or changes in the neoplastic status of these lesions in relation to molecular events. Persons with white and or red oral mucosal lesions seen at six Veterans Affairs hospitals will be selected for the study. Study subjects will undergo a biopsy of all suspect oral lesions, provide information about tobacco, alcohol intake, and other habits and will be re-examined every four months for two years and re-biopsied as appropriate. The biopsy material will be analyzed for the presence or activity of certain molecules thought to be associated with the malignant phenotype including p53-dependent growth suppressors, pRb-dependent growth control factors, growth factor receptor-modulated pathways, and viral agents. Statistical analysis will determine associations between molecular markers from the oral lesions and the lesions' histopathology and will examine how environmental, behavioral, and sociodemographic factors influence these associations. If molecules indicative of early transformation can be identified, they may be used to identify persons who should be monitored most closely for the development of oral cancer and to recognize oral cancers at the earliest, most treatable stage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00606-02 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Determination of Serum Antibodies to Periodontal Pathogens in the U.S. Population

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Marcus, Stephen E.

Senior Epidemiologist, ASHAB

DEODP, NIDR

Winn, Deborah M.

Chief, ASHAB

DEODP, NIDR

Brown, L. Jackson

Director

DEODP, NIDR

COOPERATING UNITS (if any)

National Center for Health Statistics, Centers for Disease Control & Prevention

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892-6401

TOTAL STAFF YEARS:

0.38

PROFESSIONAL:

0.31

OTHER:

0.07

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The primary purpose of this research project is to determine the occurrence of elevated serum antibodies to the periodontal pathogens *Actinobacillus actinomycetemcomitans* (A.a.) and *Porphyromonas gingivalis* (P.g.) in a normal population. The study population is comprised of persons aged 13+ examined in Phase I of the Third National Health and Nutrition Examination Survey (NHANES III). Serum from the NHANES III subjects, which has already been collected, frozen, and stored, will be assayed by ELISA procedures. The ELISA offers the benefit of quantification of antibody titers; values of 100 EU or greater for A.a. and 20 EU or greater for P. gingivalis will be employed as threshold values for designating elevated levels. The prevalence of elevated antibody titers to these organisms will be described for the entire sample and stratified by age, gender, race, and geographic region. Systemic antibody titers also will be correlated with the clinical periodontal status of study participants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE00615-02 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methodology and Analytic Development of NHANES III, Phase I

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M	Chief, ASHAB	DEODP, NIDR
Oldakowski, Richard J.	Chief, SPU, ASHAB	DEODP, NIDR
Brunelle, Janet A.	Statistician (Health), ASHAB	DEODP, NIDR
White, B. Alexander	Senior Dental Research Investigator, ASHAB	DEODP, NIDR
Kaste, Linda M.	Senior Staff Fellow, ASHAB	DEODP, NIDR
Marcus, Stephen E.	Senior Epidemiologist, ASHAB	DEODP, NIDR
Streckfus, Charles F.	Senior Staff Fellow, ASHAB	DEODP, NIDR
Kleinman, Dushanka V.	Deputy Director,	OD, NIDR
Furman, Larry	Senior Dental Epidemiologist, ASHAB	

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.75

PROFESSIONAL:

2.60

OTHER:

1.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
(a1) Minors
(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The National Health and Nutrition Examination Survey (NHANES III) was conducted by the National Center for Health Statistics (NCHS) from 1988 to 1994. NIDR staff contributed to and coordinated an oral health component. There were four overall objectives of the oral health component of Third National Health and Nutrition Examination Survey (NHANES III), Phase I (1988-1991) and Phase II (1992-1994): (1) to estimate the magnitude and relative frequency of selected oral diseases, disorders, and conditions; (2) to describe the distribution of these oral conditions among sociodemographic groups; (3) to describe the relationship between these conditions and selected risk factors; and (4) to document and investigate reasons for secular trends in these oral diseases, disorders, and conditions.

Division staff designed the oral health component examination procedures and household questions and monitored the data collection phase. Large, complex sample surveys offer many unusual challenges for analysts and computer programmers in addition to the volume of data involved. These complexities include: the need for special software, custom programming, and procedures for calculation of estimates and standard errors that take complex sampling design into account; the difficulties in ensuring that data from national surveys conducted over time are comparable; and the need for procedures to control for differences in population structure across race or ethnic groups. Intra-divisional teams create oral health status indices, develop and execute statistical analysis plans, resolve statistical and quality control issues, and interpret the findings.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00616-02 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Case-Control Study of Oral and Pharyngeal Cancers in Puerto Rico

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.	Chief, ASHAB	DEODP, NIDR
Kleinman, Dushanka V.	Deputy Director	OD, NIDR
Diehl, Scott R.	Chief, MEDIB	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.22

PROFESSIONAL:

0.17

OTHER:

0.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Puerto Rico experiences high rates of oral and pharynx cancer. This study aims to identify sociodemographic, behavioral, nutritional, dental, and occupational risk factors for oral and pharynx cancer in Puerto Rico. Another purpose is to evaluate the role of human papilloma virus and biomarkers for malignancy and malignant transformation in biological specimens from cases and controls. This study has a population-based case-control design with more than 500 cases ascertained from the cancer registry in Puerto Rico. The more than 600 control subjects are identified using two sampling frames providing good coverage and representation of the general population: a geographic area frame and Medicare rosters. Case and control subjects are interviewed in their homes using a standardized questionnaire which obtains data on a wide range of risk factors. Medical record data is being abstracted on cases. Biological specimens are being obtained from selected study subjects. Specimens include a blood sample, a buccal cell scraping, a urine sample, and pathologic slides. With collaboration of several academic and commercial laboratories and experts, NIDR and NCI have been evaluating methods for collecting buccal scrapings and laboratory methods for evaluating the presence of human papillomavirus in buccal scrapings. Data will be analyzed to determine differences between cases and controls in potential risk factors and in the presence and levels of viruses and molecular markers for malignancy. This study will provide insights into the etiology of oral and pharyngeal cancer, and the reasons for the high incidence rates of these cancers in Puerto Rico.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00618-02 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Health Assessment Databases and Public Use Data File Documentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Winn, Deborah M.	Chief, ASHAB	DEODP, NIDR
Oldakowski, Richard J.	Chief, SPU, ASHAB	DEODP, NIDR
Brunelle, Janet A.	Statistician (Health), ASHAB	DEODP, NIDR
Marcus, Stephen E.	Senior Epidemiologist, ASHAB	DEODP, NIDR
Webb, Kimberly W.	Statistical Assistant, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Health Assessment Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.50

PROFESSIONAL:

0.35

OTHER:

0.15

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

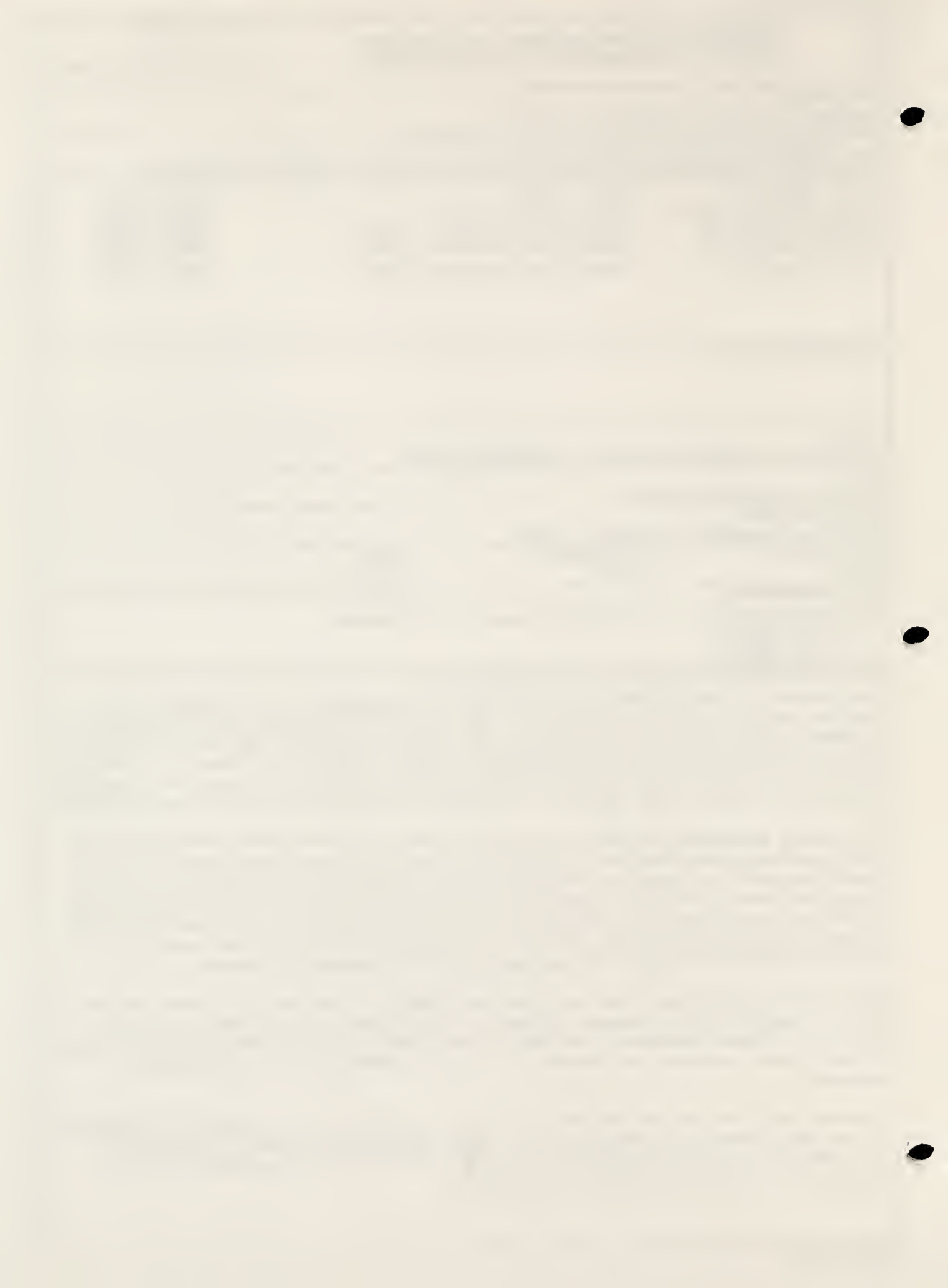
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to develop well-documented edited databases for various national oral health surveys. Develop well-documented edited databases from various national oral health surveys, e.g., the Dental Component of the Third National Health and Nutrition Examination Survey (NHANES III), Phase I (1988-1991) and Phase II (1991-1994), and the National Institute of Dental Research's (NIDR's) National Surveys of Oral Health in U.S. School Children in 1979-1980 and 1986-1987.

Developing databases involves a sequence of steps. These include development of error-checking specifications and file documentation procedures; translation of specifications into computer language code; development of standardized variable names and summary variables; examination of distributions and relationships among variables to evaluate data consistency; addressing statistical and methodological issues related to integrating data from previous NIDR surveys; and preparation of extensive documentation on the survey design and database structure.

Since 1992, public use files have been developed for the NIDR's National Surveys of the Oral Health of U.S. School Children in 1979-1980 and 1986-1987, adding to public use tapes previously developed by the Institute. Currently public use tapes of the dental component of NHANES III are being developed and documentation prepared.

The data will be used to contribute to ongoing studies on the magnitude, severity, scope, and interrelationships of oral health conditions in the U.S. population. The documentation and file structure permits researchers to perform their own analyses with minimal technical assistance.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00651-01 ASHA

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Differences in Fluoride Exposure and Expression in Adolescents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

Brunelle, Janet	Statistician (Health), ASHAB	DEODP, NIDR
Kaste, Linda M.	Senior Staff Fellow, ASHAB	DEODP, NIDR
Winn, Deborah M.	Chief, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

Indiana University

LAB/BRANCH

Analytical Studies and Health Assessment Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.07

PROFESSIONAL:

0.07

OTHER:

0.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

This study is being undertaken to better explain the decreases in dental caries prevalence in non-fluoridated areas and to determine reasons for any increases in dental fluorosis. The study will include the collection of comprehensive data on exposure to fluoride from all sources by children and adolescents.

Two communities located in a geographic region classified as a "high fluoridation" area -- one with a central water supply which is "fluoride deficient" (<0.3 ppm fluoride) and one which has been optimally fluoridated continuously since 1980 or earlier--will be selected for study. Within each community approximately 400 (ages 12 and 13 years) life-long residents will be asked to participate in the study. These subjects will receive a clinical oral examination for dental caries and enamel fluorosis. Samples of plaque, saliva, blood and urine will be collected from the subjects and evaluated for their fluoride concentrations in the laboratory. All subjects will receive a dietary collection instrument and a detailed questionnaire on residence and fluoride-use history, as well as sociodemographic, and behavioral information. In addition, water samples will be collected from community sites and school buildings for analysis; and frequently consumed foods, as listed in the dietary histories, will be purchased locally and analyzed for fluoride content. Fluoride distribution in body fluids and tissues will be correlated with each other and with reported intake, clinical findings of dental enamel fluorosis, and dental caries experience. These relationships will be evaluated between the communities and between groups of individuals with different fluorosis manifestations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00577-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevention of Dental Caries

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Selwitz, Robert H.	Dental Epidemiologist	DEODP, NIDR
Nowjack-Raymer, Ruth E.	Public Health Research Specialist	DEODP, NIDR
Horowitz, Alice M.	Education Specialist	DEODP, NIDR
Gift, Helen	Chief, DPHP Branch	DEODP, NIDR
Small, John	Public Health Advisor	DEODP, NIDR
Chang, Duk-Soo	Guest Researcher	DEODP, NIDR
Kaste, Linda	Senior Staff Fellow, ASHAB	DEODP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

American Dental Association, Forsyth Dental Center

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.27

PROFESSIONAL:

1.12

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although the overall prevalence of dental caries has declined over the past few decades in the United States, the disease still is responsible for the loss of more teeth, at all ages, than any other oral condition; it remains a problem for many subpopulations of infants and young persons and a growing number of dentate older Americans. DPHPB staff have focused efforts on investigating fluorides and dental sealants, feeding behaviors, and science transfer for enhancing the prevention of dental caries. A study in Nelson County, VA demonstrated the effectiveness of the combined use of fluoride therapy and dental sealants. A background paper for a workshop on guidelines for sealant use reviewed recent changes in the epidemiology of dental caries and assessed their potential impact on the diagnosis and management of the disease and the planning and operation of sealant programs. Analysis of other available data by staff suggest the need to continue efforts toward improving knowledge of oral disease preventive approaches and the proper use of preventive methods. NIDR organized an Ad Hoc working group to review what research activities are being undertaken in the area of infant caries, to identify research needs, and to recommend priorities for future Institute efforts. In addition, staff are involved in a pilot, community-based study of the prevention of caries among Hispanic infants and young children. Analyses of feeding behaviors data from the 1991 NHIS has been published in Archives of Pediatric and Adolescent Medicine.

[The text on this page is extremely faint and illegible. It appears to be a multi-paragraph document with several lines of text per block. The content is not discernible.]

CONTINUATION:

Z01DE00577-03 DPHP

PRINCIPAL INVESTIGATOR

Zion, Gary Computer Specialist DEODP, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00578-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Fluoride Accumulation and Effects in the Body

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Selwitz, Robert H.	Dental Epidemiologist	DEODP, NIDR
Nowjack-Raymer, Ruth E.	Public Health Research Specilist	DEODP, NIDR
Kingman, Albert	Chief Statistician, OD	DEODP, NIDR
Horowitz, Alice M.	Education Specialist	DEODP, NIDR

COOPERATING UNITS (if any)

American Dental Association, Eastman Dental Center, National Center for Health Statistics

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.36

PROFESSIONAL:

.26

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fluoride accumulation and its effects in the body and interrelations between dental caries, dental fluorosis, and the appropriate use of fluoride are important issues being addressed by staff of the DPHPB. Staff have published two manuscripts regarding the assessment of dental fluorosis in child populations having differing levels of exposure to fluoride through drinking water, dietary fluoride supplements, and other sources. DPHPB staff prepared a paper for presentation at an American Dental Association sponsored workshop on dietary fluoride supplements. In addition, staff have engaged in the dissemination of current information regarding the appropriate use of fluorides through papers delivered at professional and other scientific meetings to students, health professionals, and government officials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00584-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Minority Oral Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.	Chief, DPHP Branch	DEODP, NIDR
Robinson, D.	Health Promotion Research Specialist	DEODP, NIDR
Horowitz, Alice M.	Education Specialist	DEODP, NIDR
Kaste, Linda	Staff Fellow	DEODP, NIDR
Drury, Thomas	Deputy Branch Chief, DPHPB	DEODP, NIDR
Witt, Cecilie	Computer Specialist	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.86

PROFESSIONAL:

.80

OTHER:

.06

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In recent years there have been numerous efforts to document the health status and associated risk factors of American minorities. Review efforts to date (by staff with input from expert consultants) have raised several concerns, an important one being the heterogeneity of oral health status characteristics within diverse minority populations. Extensive literature reviews have been conducted to assess needs for additional research in Hispanic communities and to evaluate appropriateness of Healthy People 2000 special target objectives. All analyses of major data sets conducted within the DEODP consider racial/ethnic differences in disease prevalence and oral health practices. For example, all analyses of the NHANES III data have included race/ethnicity comparisons.

As more fully described in another project summary, DPHPB staff is working with a Hispanic population to study health behaviors of mothers and young children.

A request for proposal was released, responses have been received and are being evaluated to conduct a feasibility study of a community-based health promotion strategy in a minority community. This study is designed to increase focus on Black and Hispanic minority children and youth and address problems of participation by these minority youth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00585-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prevention of Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Horowitz, Alice M. Education Specialist DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.29

PROFESSIONAL:

.19

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Oral soft tissue lesions, precancers and cancers are among the most serious, and in the case of oral cancer life-threatening, oral conditions. The risk factors for many of these conditions are clearly identified as comorbidities with medical conditions and treatments and with high risk behaviors such as tobacco and alcohol use. To that end, these conditions and the risks leading to them are clear targets for health promotion. The Branch is actively pursuing research which identifies correlates of knowledge, opinions and practices associated with these oral conditions and brings focus to intervention research which could improve strategies to reduce the incidence and prevalence of these conditions and associated risk behaviors. Staff have analysed the 1990 and 1992 NHIS data regarding knowledge of the risks, signs, and symptoms of oral cancer and having had oral cancer screening examinations. These analyses were the basis of a manuscript published in JADA. Staff are collaborating with a group of researchers at the University of Maryland regarding a statewide study of the knowledge, opinions and practices of the public and health care providers regarding oral cancer, survey of dental and medical schools regarding assessing risk factors of patients for oral cancer. Staff have been active in interagency forums on oral cancer and associated risk behaviors which have been held to identify research and program needs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00586-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Women's Oral Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann

Public Health Specialist

DEODP, NIDR

Gift, Helen C.

Chief, DPHPB

DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.06

PROFESSIONAL:

.06

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Women's health has become a major priority for research at NIH. Branch staff have participated with other NIH scientists in developing clinical trial and community demonstration research agendas for women's health. Extensive literature reviews have been conducted to assess the interaction of oral health with systemic health and to determine how oral health fits within the contexts of these larger research initiatives. Projects have been developed from these efforts and now have independent project descriptions: (1) evaluating the utility of oral biomarkers for systemic diseases and conditions using saliva as a matrix for the biochemical validation of fat intake among women enrolled in the Women's Health Trial Minority Feasibility Study; (2) an investigation of oral hard tissue status in relation to skeletal bone mineral density measures and osteoporosis on a subsample of women enrolled in the observational component of the Women's Health Initiative at a designated bone density center; and (3) the oral manifestations of HIV infection in women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00587-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Quality of Life

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.	Chief, DPHP Branch	DEODP, NIDR
Redford, Maryann	Public Health Specialist	DEODP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.14

PROFESSIONAL:

.14

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research program represents a series of projects undertaken to describe and then quantify the functional, psychological, and social consequences of oral disorders and their treatment. Extensive literature reviews and consultations with external experts have been conducted in an effort to improve the measurement and interpretation of oral quality of life. Staff have worked with other agencies to improve ways of determining disability in relation to the oral cavity.

A DPHPB staff serves as project officer for an interagency agreement with the Boston VA. The intent of the project is to analyze the longitudinal data set focusing on the interaction of oral health and quality of life.

Building on the background paper for the VA Research Agenda Conference, staff are using existing data to investigate the appropriateness of quality of life models for oral health. Oral diseases and conditions are highly prevalent and the progressive consequences of these are not only physical, but economic, social, and psychological. The relation of oral health to overall quality of life has gained increasing recognition as an important area of scientific investigation. These investigations demonstrate that oral problems can alter an individual's self-image, activities, and life choices.

Results of this research initiative should improve understanding of oral health quality of life and will be useful for the development of process and outcome measures in future investigations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00588-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone Loss in the Oral Cavity and its Relation to Skeletal Bone Health

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann

Public Health Specialist

DEODP, NIDR

COOPERATING UNITS (if any)

Office of the Director, NIH

University of Alabama of Birmingham

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.40

PROFESSIONAL:

.30

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bone loss in the oral cavity is a significant problem in the United States. In the dentate, oral bone loss may manifest as a loss of tooth support. In edentate individuals, osteopenia may augment local anatomic, biological, and mechanical factors resulting in extensive ridge atrophy. There have been speculations in the medical and dental literature that generalized skeletal osteopenia may be conducive to accelerated loss of oral bone. Thus, skeletal osteopenia may influence the need for and outcome of periodontal, pre-prosthetic, and implant surgical procedures.

The objective of this research is to describe changes in oral bone mass and quality with age and in relation to skeletal bone status in health and disease. In accord with the research objectives, investigators from a designated Bone Density Center must recruit 1000 peri- and post-menopausal women enrolled in the observational component of the Women's Health Initiative (WHI). At baseline and at 3 year intervals as dictated by the WHI study protocol, participants will receive a thorough oral examination, including standardized radiographs of the posterior teeth or corresponding edentulous spaces. Interim information on self care will be obtained by querying participants and their dental providers will be asked to provide yearly updates of patient records.

Data from the oral component will be linked to the WHI participant's file thereby establishing the framework for descriptive and analytic analyses of tooth and oral bone status in relation to health behaviors, medical status, and skeletal bone density assessments.

There is a dearth of information describing changes in oral bone mass and quality with age, and in relation to skeletal bone status in health and disease. Knowledge of an osteoporotic influence on oral bone may be of particular relevance to women with regards to the need for, and outcome of periodontal, preprosthetic and implant surgical procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00589-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Orofacial Trauma

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.	Chief, DPHP Branch	DEODP, NIDR
Nowjack-Raymer, Ruth	Public Health Research Spec.	DEODP, NIDR
Witt, Cecilie	Computer Specialist	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.33

PROFESSIONAL:

.25

OTHER:

.08

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The consequences of orofacial trauma can be among the most permanent of oral conditions and diseases. Trauma (as a consequence of injuries to the face and mouth resulting from falls, sporting activities or abuse) and its prevention have recently received more attention in research. A portion of the 1991 NHIS contained questions about participation of children and youth in sports and their use of head and mouth protection. A manuscript of the findings has been accepted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00590-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluating Oral Health Status As It Relates to Problematic Eating Behaviors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann Public Health Specialist DEODP, NIDR

COOPERATING UNITS (if any)

University of California, San Francisco

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.12

PROFESSIONAL:

.12

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The nutritional status of the elderly has been studied extensively but little is known about how oral health status relates to problematic eating behaviors among nursing home residents. Because of a dearth of information, dentistry is often excluded from inter-disciplinary strategies for treating dysphagia. If oral health problems are identified as contributing to malnutrition, weight loss, or the eventual placement of a feeding tube, then appropriate oral health interventions may improve the quality of life for nursing home residents.

Literature reviews have been conducted to identify research questions and hypotheses. Collaborations have been established with extramural scientists as preparation for investigations in this area. Data collection for an initial project examining the role of oral health status in the development of problematic eating disorders among nursing home residents has recently been completed. Oral health data is being linked to relevant clinical, social, cultural, and environmental data which are part of a parent study being conducted by an extramural collaborator at the University of California, San Francisco. Interpretation of the results of this study should augment the rationale for improved recognition and treatment of presenting dental needs within this underserved population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00592-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research on Oral Health Education and Health Promotion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Horowitz, Alice M.	Education Specialist	DEODP, NIDR
Small, John S.	Public Health Advisor	DEODP, NIDR
Gift, Helen C.	Chief, DPHP Branch	DEODP, NIDR
Nowjack-Raymer R.	Public Health Research Specialist	DEODP, NIDR
Chang, Duk-Soo	Guest Researcher	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Oral health education is an integral part of health promotion. Research on ways to improve content, channels of distribution, appropriateness as well as the need to identify specific audiences and to identify their needs is a primary focus of the Branch. Science transfer activities to help ensure that research based information is used in preparation of education materials and other communications also is integral to Branch activities. Adapting research findings to the needs of the audience, be they legislative, professional, general public or specific high risk populations is an important outcome of research conducted by NIDR. The Branch takes the NIDR lead in evaluating scientific literature for translation and interpretation for the varied audiences. The outcomes of these scientific evaluations result in published literature reviews in texts and journals, lectures and consultation. Major areas of emphasis are community water fluoridation, multiple modalities of fluoride, dental sealants as well as prevention of other oral diseases such as oral cancer and HIV oral manifestations and conditions, and strategies for effective health education and promotion.

Staff have been working with outside investigators to develop a survey instrument to evaluate knowledge, opinions and practices for use in determining the basis upon which education and preventive regimens could be established at the local and state levels. Staff also have been working with an outside investigator to evaluate the content of health education text books to determine the extent, nature and appropriateness of oral health information being taught and how this information supports oral health objectives in Healthy People 2000. The Branch is working with three international guest scientists in the areas of health education and health promotion. One is analyzing a survey from Korea on knowledge, opinions, and practices of health care providers regarding caries prevention to establish the basis for a health education-promotion program.

THE HISTORY OF THE CITY OF BOSTON

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00593-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

International Health Promotion, Disease Prevention, and Epidemiologic Research

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Horowitz, Alice M.	Education Specialist	DEODP, NIDR
Nowjack-Raymer, Ruth E.	Public Health Research Specialist	DEODP, NIDR
Kleinman, Dushanka V.	Deputy Director	OD, NIDR
Small, John	Public Health Advisor	DEODP, NIDR
Chang, Duk-Soo	Guest Researcher	DEODP, NIDR

COOPERATING UNITS (if any)

World Health Organization

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.59

PROFESSIONAL:

.44

OTHER:

.15

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Numerous international initiatives which focus on improved disease prevention/health promotion research and enhanced science transfer internationally exist and include collaboration with investigators of the WHO International Collaborative Study II; authorship of articles for international publication; participation in international meetings as presenters and invited speakers, and as technical consultants and development of material. Additionally, numerous guest researchers have been hosted by and have collaborated with staff in the development of research protocols for the initiation of studies. Scientific interchange has also been facilitated through the coordination of seminars, lecturers, meetings for international guests and visitors with a broad range of agencies, organizations and universities. Health education materials developed by NIDR have been made available to international organizations and agencies to enhance rapid science transfer.

The subject matter of the international initiatives cover diverse public health topics which include caries prevention, dietary fluoride supplements for preschool age children, community water fluoridation, fluoride mouthrinse, prevention of oral cancer, infection control, HIV/AIDS prevention and control curriculum development focused on oral disease prevention, role of oral health professionals in oral disease prevention and health promotion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00595-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research Toward Preventing Periodontal Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Nowjack-Raymer, Ruth E.	Public Health Research Specialist	DEODP, NIDR
Kingman, Albert	Statistician (Health)	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Disease Prevention Section

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.16

PROFESSIONAL:

.13

OTHER:

.03

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Self care remains the most effective approach for the prevention of periodontal diseases. Several studies of the Disease Prevention and Health Promotion Branch address the prevention of periodontal diseases: a study of two different school-based approaches to prevent gingivitis in teenagers; and 3) analysis of NHANES III data.

A two year study of teenagers was conducted in York County, Virginia to determine the effectiveness of a self-assessment of gingival bleeding approach to the prevention of gingivitis compared with a plaque control approach. Both the plaque control and self-assessment of bleeding groups received an interactive manual describing the procedures they were to perform, classroom-based instruction and individual instruction specific to their needs. The individual instruction was reinforced following a 12 month interim oral examination. Findings show that while the two approaches did not differ, there were improvements in the gingival health status of both groups improved with over a 50% reduction in the mean number of gingival bleeding sites for both groups. A manuscript has been accepted for published. Additional analysis is underway.

Ongoing consultation and assistance is provided for the planning and development of research conferences, symposia and research groups that focus on research related to periodontal disease prevention and oral health status improvement. Presentations are made as a part of science transfer efforts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00597-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Healthy People 2000

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gift, Helen C.	Chief, DPHP Branch	DEODP, NIDR
Horowitz, Alice M.	Education Specialist	DEODP, NIDR
Nowjack-Raymer, R.	Public Health Research Spec.	DEODP, NIDR
Selwitz, Robert	Dental Epidemiologist	DEODP, NIDR
Small, John S.	Public Health Advisor	DEODP, NIDR
Drury, Thomas	Deputy Chief, DPHP Branch	DEODP, NIDR
Brunelle, Janet	Statistician	DEODP, NIDR
Oldakowski, Richard	Chief, SPU, ASHAB	DEODP, NIDR

COOPERATING UNITS (if any)

Centers for Disease Control
Chief Dental Office

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.50

PROFESSIONAL:

.39

OTHER:

.11

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

NIH is the co-lead agency for the oral health objectives and in that role assumes major responsibility for research to: 1) monitor progress and 2) establish relevant research programs to help achieve objectives. Oral health is one of 22 primary areas in Healthy People 2000, the nation's objectives for the 1990's. There are 13 objectives and dozens of sub-objectives. The Health Promotion and Disease Prevention Branch 1) provides leadership and the research basis for ongoing monitoring, planning and formal review of national progress on the Healthy People 2000 Oral Health Objectives, and 2) fosters or conducts appropriate research to help achieve the objectives and provides the scientific basis for these activities. Multiple methods are used including: analyses of existing data, initiation of new research investigations, development of new and appropriate measurement and analytic approaches, such as surveys, focus groups, and demonstration projects. Branch staff serve on working groups, prepare reports and represent NIH on all Healthy People 2000 activities. All reports are prepared for the DHHS Assistant Secretary, the senior policy staff for health in the U.S., and require extensive analyses of existing data and reviews of literature to ensure scientifically based responsiveness. In an effort to extend science transfer to the broader community, staff have developed papers related to objectives in Healthy People 2000 for publication in professional journals, and have worked with NCHS staff in the development of measures for upcoming surveys that will improve assessment of the objectives. Staff also have conducted data analysis to monitor objectives for the mid-course review. Many presentations and publications cited in staff CVs are related to Healthy People 2000.

Staff coordinated and implemented a briefing with the Assistant Secretary for Health in July 1995. Representatives of the public health sector and professional associations participated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00596-03 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Orofacial Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Drury, Thomas	Deputy Branch Chief, DPHP	DEODP, NIDR
Robinson, Dina	Health Promotion Research Specialist	DEODP, NIDR

COOPERATING UNITS (if any)

Other NIH Institutes; expert consultants

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

INSTITUTE AND LOCATION

NIDR,NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.32

PROFESSIONAL:

.32

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data analysis and interpretation of orofacial pain data on existing surveys continue.

Section 1907 of the National Institutes of Health Revitalization Act of 1993 has requested that the Director of the National Institutes of Health (NIH), acting through the Director of the National Institute of Dental Research (NIDR) and through the heads of other NIH agencies, conduct a study of the frequency and health care costs of chronic pain conditions in the United States. The results of this study are to be submitted in a Report to Congress. The focus of this study is on the following selected chronic pain conditions: (1) chronic low back pain, (2) reflex sympathetic dystrophy (RSD) syndrome, (3) temporomandibular (TM) joint and muscle disorders, (4) posttherapeutic neuropathy, (5) painful diabetic neuropathy, (6) phantom pain, (7) post-stroke pain. The objectives of this study involve four evaluations: (1) of existing classifications, case definitions, diagnostic criteria, and case ascertainment procedures in the context of the phenomenology and clinical spectrum of these conditions and associated health care utilization, (3) of what is known about the direct medical care costs of these conditions, and (4) of the current state of the epidemiologic and economic science of chronic pain from the perspectives of basic, clinical, population, health services, and economic research. To achieve these objectives a series of background papers and reviews has been commissioned. These papers and reviews will be discussed at a Workshop in FY 1996 in the Greater Washington, D.C. area. An analytical summary of these papers will provide the basis of the Report to Congress. In April, 1995, due to scheduling conflicts, this congressionally-mandated study was transferred to the Office of Planning, Evaluation and Coordination, Office of the Director, NIDR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00613-02 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NHANES III-PHASE I Analyses: Coordination and Team Leadership and Participation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Drury, Thomas	Deputy Branch Chief, DPHPB	DEODP, NIDR
Gift, Helen C.	Chief, DPHPB	DEODP, NIDR
Nowjack-Raymer R.	Public Health Research Specialist	DEODP, NIDR
Selwitz, Robert H.	Research Dentist	DEODP, NIDR
Horowitz, Alice M.	Education Specialist	DEODP, NIDR
Redford, Maryann	Public Health Specialist	DEODP, NIDR
Robinson, Dina	Health Promotion Research Specialist	DEODP, NIDR

COOPERATING UNITS (if any)

National Center for Health Statistics

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.89

PROFESSIONAL:

.84

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the past 12 months, DPHP staff have worked cooperatively and provided leadership in coordinating Division activities regarding the analysis of oral examination data from the first three years of the 1988-1994 National Health and Nutrition Examination Survey (NHANES III-Phase I). Analytic teams met on a regular basis to prepare formal analysis plans, review analyses and prepare final manuscripts. Branch staff contributed to a series of initial analyses for a special issue of the Journal of Dental Research to be published early in 1996. Topics include tooth loss, edentulism, quality of protheses, orofacial trauma, dental caries, tooth conditions and restorative treatment needs, and methodology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00617-02 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Manifestations of HIV in Women

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann	Public Health Specialist	DEODP, NIDR
Nowjack-Raymer, Ruth	Public Health Research Specialist	DEODP, NIDR
Brunelle, Janet	Statistician	DEODP, NIDR

COOPERATING UNITS (if any)

National Institute of Allergy and Infectious Diseases
National Institute of Child Health and Human Development
Centers for Disease Control and Prevention

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.34

PROFESSIONAL:

.29

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Seemingly little attention had been paid to human immunodeficiency virus (HIV) infection in women. Much of what is currently known about HIV disease has been learned through the prospective study of large cohorts of gay men. Investigative efforts in the dental community have paralleled those in the general medical field. Most reports appearing in the dental scientific literature regarding the oral manifestations of HIV disease in women do not discuss how they relate to hormonal status and correlate with genital manifestations.

Recognizing that the multisite Women's Interagency HIV Study (WIHS) is poised to break scientific ground relative to HIV infection in women, NIDR branch staff have taken the leadership role in developing an oral component that includes data collection about the oral manifestations of HIV disease in women, particularly as they relate to hormonal status and correlate with genital manifestations. Significant participation of the NIDR in this collaborative effort provides an efficient, cost-effective and timely means targeting research towards several high-risk populations - women, minorities, and HIV infected individuals.

Staff in the DPHPB is providing leadership for protocol design and conduct of the oral component. NIDR sponsored training for this study which was conducted in February, 1995. Baseline oral examinations began in April 1995 and are presently underway.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00653-01 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assessment of Saliva as a Matrix for the Biochemical Validation of Fat Intake

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Redford, Maryann

Public Health Specialist

DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

Health Promotion Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.20

PROFESSIONAL:

.15

OTHER:

.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In recent years epidemiologists and other medical researchers have become increasingly interested in the effects that diet may have on health and disease. The chief impediment to research on nutritional causes of disease has been uncertainty about the validity of existing dietary assessment methods and the consequent uncertainty about the results obtained from them. Ascertainment of fat intake by means of salivary analyses affords an attractive alternative to more conventional procedures which are either subjective or invasive. Positive outcomes along this line of investigative research will facilitate the conduct of valid and useful research on various chronic diseases, including cancer and heart disease.

This is a prospective study exploring the utility of salivary lipid profiles as an adjunct to more traditional dietary assessment methods among women enrolled in the Women's Health Trial (WHT) Feasibility Study in Minority Populations. Study volunteers were followed in the central study but in addition donated saliva at baseline, 3 month and 6 month follow-up. The collected saliva specimens were stored and shipped to the analytic laboratory at Texas A & M University for lipid analyses. All data from the salivary component will be collated with pertinent data from the Women's Health Trial in order to evaluate the associations among salivary lipids, blood lipids, dietary intake data, and other pertinent variables.

The role of nutrition on health and risk of chronic diseases is gaining increasing attention as a potential modality for the prevention of disease. Currently available techniques for assessing dietary intake have major limitations: reliance on self-reported data, inaccuracies in reported food portion, content and preparation methods. The use of salivary lipids to assess dietary intake and adherence to low-fat diets would greatly aid the conduct and interpretation of clinical trials involving fat intake.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00658-01 DPHP

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

1996-1997 National Survey of the Oral Health of U.S. School Children (OHSC III)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Drury, Thomas	Deputy Branch Chief, DPHP (Project Officer)	DEODP, NIDR
Kaste, Linda M.	Staff Fellow, ASB (Co-Project Officer)	DEODP, NIDR
Nowjack-Raymer, R.	Public Health Research Spec. (Co-Project Officer)	DEODP, NIDR
Selwitz, Robert	Research Dentist, (OHSC III Dental Consultant)	DEODP, NIDR
Brown, L. Jackson	Division Director	DEODP, NIDR
Gift, Helen C.	Chief, DPHPB	DEODP, NIDR
Winn, Deborah M.	Chief, ASB	DEODP, NIDR
Kingman, Albert	Chief Statistician	DEODP, NIDR
Brunelle, Janet	Senior Health Statistician, ASB	DEODP, NIDR

COOPERATING UNITS (if any)

Other NIDR Divisions; expert consultants

LAB/BRANCH

Disease Prevention and Health Promotion Branch

SECTION

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.09

PROFESSIONAL:

1.05

OTHER:

.04

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project entails the design and implementation of the 1996-1997 National Survey of the Oral Health of U.S. School Children (OHSC III). The major objectives of OHSC III are threefold: (1) to assess the relative frequency and sociodemographic distribution of certain oral diseases and disorders in U.S. schoolchildren in grades K through 12, (2) to oversample selected minority schoolchildren to provide statistically reliable baseline national estimates of oral health for Black non-Hispanic and Hispanic schoolchildren, and (3) to provide the database for the late nineties needed to evaluate shorter- and longer-term trends in coronal caries and certain other oral diseases and disorders. Oral examinations will be taken with a national scientific sample of approximately 31,000 U.S. schoolchildren. The oral examinations will yield information on clinical parameters of coronal caries, gingivitis, periodontal diseases, selected occlusal and craniofacial characteristics. Parental and student questionnaires will yield information on sociodemographic characteristics, oral health behaviors, and oral-health related information on community contexts. The main data collection will take place during the 1996-1997 academic school year. Methodological and substantively-oriented analyses will be carried out during 1997-1999.

During this past fiscal year, the RFC for the project was developed, an RFP was announced, responses to the RFP were evaluated by an extramural review panel, a contract was negotiated and awarded, and materials for the Office of Management and Budget clearance package were developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00622-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Mapping of Early Onset Periodontitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB	DEODP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	DEODP, NIDR
Sun, Cathy	MGES, MEDIB Branch	DEODP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	DEODP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	DEODP, NIDR
Gregg, Mary	SGES, MEDIB Branch	DEODP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	DEODP, NIDR
Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

Schenkein, H. Medical College of Virginia
Lopez, N. Chile

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.05

PROFESSIONAL:

4.05

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies suggested that genetic variation in the HLA region of chromosome 6p may influence susceptibility to early onset periodontitis (EOP). Results of segregation analyses support the possibility that risk of EOP may be due to a single major gene. We conducted linkage analyses to evaluate the hypothesis that a gene within the HLA region significantly contributes to risk of EOP. Fifty families, with two or more close relatives affected by EOP, were ascertained in Virginia, USA and Chile. DNA was extracted from blood and a highly polymorphic marker located within the HLA region (near the Tumor Necrosis Factor Beta locus) was typed using the polymerase chain reaction. Linkage analyses were performed using a dominant model of disease transmission which is most strongly supported by previous studies. For the dominant model, assuming that EOP is a homogeneous disorder, our results statistically exclude the hypothesis that a susceptibility gene lies within 10cM (approximately 10 million bases of approximately 0.5% of the human genome). Additional analyses are planned for alternative modes of disease gene transmission. Under the assumption that EOP may actually consist of several etiologically distinct diseases having very similar clinical presentations our data still provide no support for HLA region involvement. However, our data do not statistically exclude (LOD <02.0) hypotheses of disease locus heterogeneity including models where up to half of our families contain a gene located in the HLA region which confers susceptibility to EOP. This is due to the limited power of even our relatively large collection of families and the inherent difficulties of mapping genes for disorders that have complex and heterogeneous etiologies. Additional statistical analyses, recruitment of families, and typing of flanking DNA markers are planned to more conclusively address these issues with respect to the HLA region and other candidate locations in the human genome.

Gene Mapping of Early Onset Periodontitis

Zhang, Yi Jie	MGES, MEDIB Branch	DEODP, NIDR
Chopra, Nandita	MGES, MEDIB Branch	DEODP, NIDR
Wang, Yue-Fen	MGES, MEDIB Branch	DEOPP, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00623-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Mapping of Cleft Lip and Palate Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB	DEODP, NIDR
Miller-Chisholm, Ann	Asst. To Chief, MEDIB	DEODP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	DEODP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	DEODP, NIDR
Gao, Yu e	MGES, MEDIB Branch	DEODP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	DEODP, NIDR
Gregg, Mary	SGES, MEDIB Branch	DEODP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	DEODP, NIDR
Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

Ballew, Carol	CDC
Mazaheri, M.	Lancaster
Long, R.	Lancaster

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.97

PROFESSIONAL:

.97

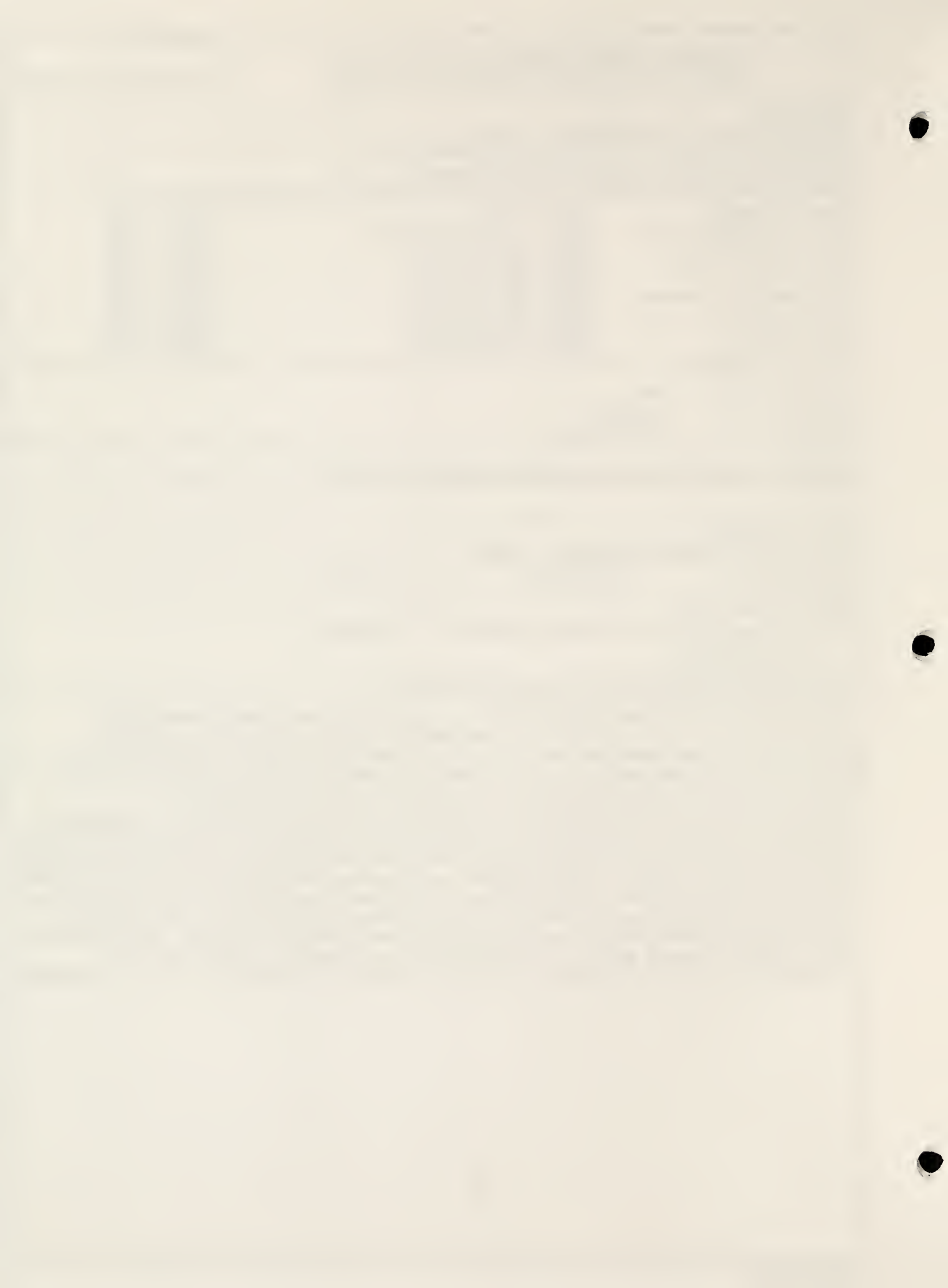
OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clefting of the lip and palate (CLP) is one of the most common craniofacial anomalies, with a rate of 1:650 in the United States population. Over 250 genetically based syndromes exist which include cleft lip or palate as a feature of their clinical presentation. In FY 1994, the NIDR initiated a collaborative research study with the Lancaster Cleft Palate Clinic in Lancaster. The Lancaster Collaborative Study of Cleft Lip and Palate (LCS) is a large scale epidemiologic study, involving the comprehensive mapping of highly informative DNA markers, utilizing the clinical population from the Lancaster Cleft Palate Clinic and affiliated clinics. Semiautomatic gene mapping technologies developed by MEDIB in ongoing CLP studies will be applied to map gene/genes responsible for clefting. The study population will consist of approximately 2300 patients and controls including 225 families with more than one child affected with CLP. DNA extracted from blood or buccal cells will be genotyped utilizing 29 panels of 12-17 microsatellite loci and genetic linkage and association analyses will be performed.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 DE00624-02 MEDIB
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gene Mapping of Kartagener Syndrome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR
Diehl, Scott R.	Chief, MEDIB	DEODP, NIDR
Miller-Chisholm, Ann	Asst. to Chief MEDIB Branch	DEODP, NIDR
Wang, Shengbiao,	MGES, MEDIB Branch	DEODP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	DEODP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	DEODP, NIDR
Gregg, Mary	SGES, MEDIB Branch	DEODP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	DEODP, NIDR
Sun, Cathy	MGES, MEDIB Branch	DEODP, NIDR
COOPERATING UNITS (if any) Witt, M. Poznan, Poland		
LAB/BRANCH Molecular Epidemiology and Disease Indicators Branch		
SECTION		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: .87	PROFESSIONAL: .87	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The immotile cilia syndrome (ICS) is a genetically determined disorder characterized by dysmotility or immotility of the cilia in airway epithelial cells, spermatozoa and other ciliated cells of the body. Kartagener syndrome (KS) is a subgroup of ICS characterized by a classic triad of symptoms: situs inversus, bronchiectasis and chronic sinusitis. Ciliary immotility is caused by various ultrastructural defects of cilia, predominantly by a lack of dynein arms. The clinical consequences of KS include pronounced craniofacial manifestations.</p> <p>In FY94 a large scale collaborative genetic epidemiology study of KS was planned with Dr. Michael Witt in Poznan, Poland.</p> <p>Over the next 12 months sixty Polish families with at least one child affected with KS will be recruited for this study. Coded DNA samples and research medical records will be sent to MEDIB, NIDR, NIH laboratory for genotyping.</p> <p>To facilitate large scale genetic mapping of the human genome we will apply microsatellite markers suitable for use with a fluorescence-based automated DNA fragment analyzer. They are arranged into 29 sets, covering 22 autosome and the X chromosome, with an average interval of 10cM. Each set consists of 12-17 marker loci, with allele size ranges that do not overlap. Marker loci were selected on the basis of their reliability in PCR, polymorphism content, map position and the accuracy with which alleles can be scored automatically by the Genotypes™ program.</p> <p>Classic pair wise linkage analyses using the program MENDEL which calculates the LOD score for complex diseases will be conducted in MEDIB. Multipoint linkage analyses will also be performed by calculating location scores using the programs LINKAGE and MENDEL.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00625-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Etiology of Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Schwartz, Joel L.

BDMS, MEDIB Branch

DEODP, NIDR

Diehl, Scott R.

CHIEF, MEDIB Branch

DEODP, NIDR

Gu, Xinbin

BDMS, MEDIB Branch

DEODP, NIDR

COOPERATING UNITS (if any)

West, K. Harvard School of Dental Medicine

Shklar, G. Harvard School of Dental Medicine

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.69

PROFESSIONAL:

.69

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lichen planus and oral leukoplakia, white lesions of the oral cavity were examined using immunohistochemical methods. The monoclonal antibodies and enzymes disclosed a dysregulation of cell growth not previously recognized in these possibly premalignant lesions. In the specific entity of erosive lichen planus we observed an abnormal expression for the p53, and the stress proteins in the oral mucosa. In the characteristic inflammatory infiltrate we noted an increase in Bcl-2 expression. Nucleosome formation was also found to be increased in the basal segment of the oral mucosa. Oral leukoplakia exhibiting dysplasia also showed high levels of p53 expression with localized areas of nucleosome formation, and Bcl-2 staining. Taken together these results indicated a profound dysregulation of cell growth and death.

Using tissues sections from a hamster buccal pouch oral mucosa treated with the carcinogen 7,12 dimethylbenz(a)anthracene (DMBA) a similar immunohistochemical study was performed using the identical monoclonal antibodies and enzymes. Early in the process of oral carcinogenesis we saw the expression of p53. and the stress proteins (70,25kD). Nucleosome formation was seen but was gradually replaced when carcinoma-in-situ was histopathologically evident, Bcl-2 was then noted in localized and expanding areas of the mucosa. In combination with markers for cell proliferation (PCNA, cell cycle) these studies indicated that oral carcinogenesis involved a suppression of programmed cell death and the normal expression of proteins such as p53 and stress proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00626-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Mapping Panels

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR
Diehl, Scott R.	Chief, MEDIB Branch	DEODP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	DEODP, NIDR
Sun, Cathy	MGES, MEDIB Branch	DEODP, NIDR
Freas-Lutz, Diana	MGES, MEDIB Branch	DEODP, NIDR
Gillanders, Elizabeth	MGES, MEDIB Branch	DEODP, NIDR
Gregg, Mary	SGES, MEDIB Branch	DEODP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

Madden, D	Applied Biosystems Div. of Perkin Elmer
Budiansky, M.	Applied Biosystems Div. of Perkin Elmer
Gilbert, D.	Applied Biosystems Div. of Perkin Elmer

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Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.05

PROFESSIONAL:

1.05

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We are evaluating 29 panels of fluorescently labeled markers located at approximately 10cM intervals. Each chromosome is covered at this marker density in 1-4 panels (11-17 loci/panel). Individual markers are labeled with 1 of 3 different fluorescent dyes, combined after PCR and run in a single gel lane. Genotypes are obtained for each locus using Applied Biosystems automated DNA Sequencer, and GENESCAN analysis, Genotypes, and Excel software. These programs automate the identification of alleles by distinguishing major peaks from PCR artifacts and facilitate the export of data in a format suitable for standard genetic analysis programs. To verify the reported genetic relationships among individuals involved in gene mapping studies, we developed software to determine the number of alleles shared among individuals within a family. We use these statistics to distinguish full and half sibs and parent-child relations from unrelated individuals. Finally, we are developing a database using Fourth Dimension software so that the tremendous amounts of data generated can be processed efficiently in an integrated suite of specialized computer programs for linkage/association studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00627-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Allele Sharing Algorithms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.

Chief, MEDIB Branch

DEODP, NIDR

Walczak, Cindy

SGES, MEDIB Branch

DEODP, NIDR

COOPERATING UNITS (if any)

Dean, M.

NCI

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.20

PROFESSIONAL:

.20

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A computer algorithm is being developed to count the number of alleles that siblings and half siblings have in common, after the mother's alleles are subtracted from each child. These allele counts are averaged for each pair of people in question. Deviations from expected average values give an indication of problems in the pedigree structure (e.g., non-paternity, mislabeled DNA samples) when a large number of genetic markers are tested. The method has been used to examine 60 JP pedigrees with favorable preliminary results. When conducting linkage analysis studies using family pedigrees, it is important that the family structure is accurately reported, in order to detect linkage between an inherited disease and genetic marker. To improve our ability to verify family structures, we have developed a new strategy to evaluate allele sharing among family members.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00628-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemoprevention in Oral Cancer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

Schwartz, Joel L.

BDMS, MEDIB Branch

DEODP, NIDR

COOPERATING UNITS (if any)

Shklar, G. Harvard School of Dental Medicine

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.30

PROFESSIONAL:

.30

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several studies were conducted to evaluate the role of chemopreventatives during oral carcinogenesis in the hamster buccal pouch tumor model. Tissue sections and cells which were treated in vivo were analyzed using immunohistochemistry, flow cytometry, and Western immunoblotting. Treatment with the carotenoid, β -carotene was administered to the hamsters producing fewer and smaller oral squamous cell carcinomas. The histopathology sections showed fewer areas of dysplasia and less invasive oral carcinomas once they did form. Results showed that nucleosome formation persisted from early to late carcinogenesis following treatment with β -carotene. Levels of Bcl-2 expression in contrast was not elevated during carcinogenesis with chemopreventative treatment. Stress proteins(70, 90) were increased very early during carcinogenesis while p53 was reduced in expression. Cell cycle was altered with the majority of the cells in G1, and showing less expression of PCNA. Treatment with either vitamin E or reduced glutathione also produced similar results. Transforming growth factor alpha and epidermal growth factor receptor expression was also noted to be reduced in staining while transforming growth factor beta was seen to be increased. Neovascularization was also apparently reduced as distinguished by fewer factor VIII antigen endothelial vascular spaces observed. These studies indicated that chemopreventative agents inhibited oral carcinogenesis by inducing programmed cell death, reducing growth factor expression, and inhibiting the development of angiogenesis. These results could lead to the development of new markers for early neoplasia identification in clinical tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00629-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Database for Genetic Epidemiological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	DEODP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	DEODP, NIDR
Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR
Wang, Yue-Fen	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

Tiller, G 4th Dimension Developer

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.50

PROFESSIONAL:

.50

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

MEDIB has begun developing, implementing and documenting a comprehensive laboratory data management system. This system will be used for the management of clinical, pedigree and genotype data for gene mapping studies. The database format shall be used for multiple databases (among them, juvenile periodontitis, cleft lip and palate and Kartagener Syndrome). The databases and programs will be implemented in 4th Dimension (4D) on Mackintosh computers. Mr. George Tiller, 4D Register Developer, is cooperating with MEDIB staff to develop appropriate procedures and documentation. The completed system will integrate and manage clinical information, family histories, and marker-allele typing as well as provide interactive procedures for 1) extensive error checking 2) generation of formatted files suitable for input to various linkage analysis programs, and 3) provide primer inventories and for tracking DNA samples and the cell lines produced from them.

Future effects will include developing procedures for 1) checking genetic incompatibilities and 2) rounding and banning allele sizes for more direct input into the database.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00630-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Analytical Programs for Genetic Epidemiological Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	DEODP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	DEODP, NIDR
Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR
Wang, Yue-Fen	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.45

PROFESSIONAL:

.45

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene mapping studies using DNA markers to detect craniofacial and oral diseases utilize a wide variety of statistical techniques. We are developing a scheme to better integrate these software tools. Since these programs were written elsewhere using a variety of computer languages and input for integrating them with our interval database will streamline the analysis process. The programs include:

- 1) MENDEL, to calculate log odds and estimate linkage between disease and genetic DNA marker loci,
- 2) affected sibling pair method
- 3) affected pedigree marker method
- 4) CRI-MAP to determine multi-point marker
- 5) HETERGEN - to test for linkage allowing for possible locus heterogeneity and calculating admixture odds LOD scores
- 6) SIMLINK - to estimate the probability or power of detecting linkage for a set of family pedigrees. This program simulates cosegregation of trait and marker loci in pedigrees resulting in an objective power calculation to determine if the collection of families is sufficient to demonstrate linkage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00631-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Epidemiological Studies of Waardenburg Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.

Chief, MEDIB Branch

DEODP, NIDR

Bock, Carla

SGES, MEDIB Branch

DEODP, NIDR

COOPERATING UNITS (if any)

Nance, W.

Medical College of Virginia

Arnoe, K.

Gallaudet

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.20

PROFESSIONAL:

.20

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We performed linkage analyses and tests of locus heterogeneity of Waardenburg Syndrome (WS) using 9 DNA markers from 2q35-37, including two highly polymorphic microsatellites very closely linked to the candidate PAX3 locus. Analysis of 14 WS Type 1 (WS1) families at PAX3 yielded a maximum LOD score of 27.81, $\theta_f = .010$, $\theta_m = .007$ assuming homogeneity. However, we found significant evidence of heterogeneity in our study, with approximately 90% of our families linked to the PAX3 region. None of five WS Type 2 (WS2) families showed linkage to the PAX3 candidate region, and linkage was excluded (LOD < -2.0) up to a distance of 17.5 cM. We localized the marker D2S102 to less than 1cM from PAX3 locus ($\theta = 0$), and thus were unable to determine whether it mapped distally or proximally due to lack of crossovers between these two markers. Meiotic breakpoint analysis in one of the three families with a crossover between WE1 and PAX3 provides strong evidence that the disease gene in this family is located elsewhere in the genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00632-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Mapping of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	DEODP, NIDR
Wang, Shengbiao	MGES, MEDIB Branch	DEODP, NIDR
Sun, Cathy	MGES, MEDIB Branch	DEODP, NIDR
Gregg, Mary	SGES, MEDIB Branch	DEODP, NIDR
Walczak, Cindy	SGES, MEDIB Branch	DEODP, NIDR
Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

Gersher, E.	NIMH
Golden, L.	NIMH
Ryerley, W.	University of Utah

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.10

PROFESSIONAL:

1.10

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Extensive genome scanning techniques using automated fluorescent microsatellite typing methods in combination with linkage analysis have been used to demonstrate evidence of linkage to Schizophrenia on chromosome 6pter-p22. The collaborative findings based on analysis of 186 multiplex Schizophrenia families have been submitted for publication. Genome scanning techniques from this collaborative effort are being applied to ongoing studies of early onset periodontitis and cleft lip and palate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00633-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mapping of Cleft Lip and Palate in the Mouse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott R.	Chief, MEDIB Branch	DEODP, NIDR
Bock, Carla	SGES, MEDIB Branch	DEODP, NIDR
Monsour, Michael	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

Erickson, R. University of Arizona

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.93

PROFESSIONAL:

.93

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The human/mouse homology map provides an excellent tool to identify candidate genes involved in human disease. Studies have been initiated using several newly identified loci in man that are candidates for involvement in facial clefting. In collaboration with Dr. Erikson, we have used markers for homologous positions in the mouse to search for QTLs affecting facial clefting. The search identified a region of mouse chromosome 3 homologous to human 1q21 as increasing liability to sporadic CL(P) when the A/J strain allele is present. This effect might be expected since the A/J strain has much higher incidence of sporadic CL9P) than does the C57B16J strain, the other progenitor strain of the RI lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00635-02 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies with Odontogenic Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Schwartz, Joel L.

BDMS, MEDIB

DEODP, NIDR

COOPERATING UNITS (if any)

Miller, G.

Navy Dental Research

Kratochvil, F.

Chairman Navy Dental Oral Pathology

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

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INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.40

PROFESSIONAL:

.30

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This laboratory has established a series of odontogenic cell lines. These cell lines are primary cell lines and include at least one of each of the following lesions: 1) ameloblastoma, 2) odontogenic fibroma, 3) myxoma, 4) keratinizing odontogenic cyst, 5) gingival fibromatosis and normal gingival fibroblasts and oral mucosa from the identical patient, and 6) normal gingival fibroblasts and periodontal ligament fibroblasts.

The cell lines have been examined by a scanning and ultrastructural electronmicroscopy. The ameloblastoma, myxoma, gingival fibroblatosis, and odontogenic fibroma have produced lesions in tumorigenicity assays with the nude mouse.

The odontogenic fibroma has also been analyzed for its protein profile, which is significantly different from the protein profiles of normal gingival fibroblasts or periodontal ligament fibroblasts. Western immunoblotting further demonstrated decreased levels of expression for minimum, chondrotinin sulfate, and N-CAM. Laminin was also decreased in expression following treatment with increased amounts of fibroblasts growth factors. Differences between the various fibroblast populations and collagen expressions, as well kinase activities were also observed to be altered. For example, the level of protein kinase C was elevated in the fibroma derived fibroblasts compared to the other populations. Treatment of the fibroma derived fibroblasts with fibroblast growth factor beta also resulted in an high level of expression for IL-1 beta, and proliferation. This laboratories further investigating a relationship between growth factor response and lymphokine production by these cell lines. These studies could provide a control of cell growth in the jaw reducing bone damage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00654-01 MEDIB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Epidemiological Studies of Nasopharyngeal Cancer (NPC)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Diehl, Scott	Chief, MEDIB Branch	DEODP, NIDR
Wang, Yue-Fen	SGES, MEDIB Branch	DEODP, NIDR

COOPERATING UNITS (if any)

Alisa Goldstein	EEB, EBP, NCI
Allan Hildesheim	EEB, EBP, NCI

LAB/BRANCH

Molecular Epidemiology and Disease Indicators Branch

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.35

PROFESSIONAL:

.35

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nasopharyngeal carcinoma (NPC) is a rare disease in most countries throughout the world (1/100,000), but it occurs at up to a 100-fold higher frequency in the Chinese people of Southern China and Southeast Asia. The high incidence also appears in immigrant Chinese. Family history is known to be a very important risk factor for NPC. To investigate the potential genetic component for susceptibility, a family study of NPC was carried out in Taiwan by Dr. Y-F Wang prior to joining the MEDIB. Detailed information about family history of cancer, smoking habits, and consumption of salted fish during childhood was collected from 750 NPC probands. Segregation analysis of a truncated trait with a logistic probability density function for age of onset was performed. Results suggest that familial clustering of NPC can be best explained by a Mendelian recessive locus with gender specific susceptibility, where consumption of salted fish during childhood and the number unaffected preceding sibs also affects risk. Under this model, the frequency of the high risk allele was 0.18, and the lifetime susceptibility of NPC was 0.09 for females and 0.18 for males with the high risk genotype. Penetrance of this putative high risk genotype by age 80 is 8% for females and 16% for males, compared to 0.04% for females and 0.07% for males with low risk genotypes. Individuals who consumed salted fish during childhood have a slightly higher penetrance (8.8% for females, and 17.2% for males by age 80 for the high risk genotype). The best-fitting model suggests that the high risk gene accounts for over 90% of NPC in this population.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00507-06 ODIR

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of HIV-1 Protease/Inhibitor Complexes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Torchia DA	Chief	ODIR, NIDR
Others:	Wang YX	IRTA Fellow	ODIR, NIDR
	Freedberg D	IRTA Fellow	ODIR, NIDR
	Hinck AP	IRTA Fellow	ODIR, NIDR

COOPERATING UNITS (if any)

PEL, (Wingfield P); NIDDK, (Bax A); DuPont Merck, (Domaille P)

LAB/BRANCH

Office of the Director Intramural Research

SECTION

Molecular Structural Biology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

3.2

PROFESSIONAL:

3.2

OTHER:

0

CHECK APPROPRIATE BOX(IES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The HIV protease is a leading candidate for targeted antiviral drug design because inhibition of this enzyme results in production of non-infectious virions. DMP323, a cyclic urea based protease inhibitor, is an attractive lead compound for an antiviral agent because of its high affinity and specificity for the HIV protease. We have determined the 3D solution structure of the protease/DPM323 complex, determined the protonation states and pKa's of its catalytic aspartyl residues, and characterized the flexibility of the protein and its interactions with solvent molecules. This work is the first comprehensive biophysical characterization of an HIV-1 protease/inhibitor complex in solution. We have also begun a detailed investigation of the protease complexed with a promising, high affinity, peptidomimetic inhibitor, KNI272, currently undergoing clinical trials at the NCI/NIH.

The significance of this project arises from the unique, detailed structural information that we are deriving about HIV protease in solution. This information will contribute improved drug design procedures based upon the understanding of the structure/function relationships of the protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00510-06 ODIR

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Cartilage Matrix Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T.I. Morales Special Expert ODIR, NIDR

Others: D. Carbott Biologist BRB, NIDR

COOPERATING UNITS (if any)

Dept. of Orthopedics, University of Pittsburgh

LAB/BRANCH

Office of the Director Intramural Research

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

In past studies, we established that interleukin-1, bacterial lipopolysaccharides and retinoic acid are strong inducers of catabolism in cultured articular cartilage and that in opposing fashion, insulin-like growth factor-1 (IGF-1) and transforming growth factor- β (TGF- β) reduce catabolism and increase synthesis of matrix components. We proposed that these effectors are part of a tightly regulated circuitry in cartilage that is flexibly modulated to direct growth, homeostasis, catabolism and/or repair. In the past year, we focused on characterizing the accessory proteins/signaling intermediates to two of the major mediators, interleukin-1 and IGF-1. We established the presence of two binding proteins of IGF-1 in bovine articular cartilage, of ~33 and 24 Kda. The first was identified as IGF-BP-2 and the second was tentatively identified as IGF-BP-4, and shown to be synthesized by the chondrocytes. We found that these proteins are present in cartilage throughout its developmental growth and we are now exploring whether these proteins play a pivotal role in directing tissue growth. In collaboration with Dr Chris Evans, we examined the role of nitric oxide in articular cartilage metabolism and found that this gas is 1) induced by interleukin-1 treatment of bovine articular cartilage organ cultures and 2) it protects cartilage from the degradative effects induced by the cytokine. We suggest that nitric oxide is a feedback inhibitor of interleukin-1 action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE00576-03 ODIR

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structural Studies of Fibronectin Modules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Torchia DA Chief ODIR, NIDR

Others: Copie V IRTA Fellow ODIR, NIDR
Akiyama S Research Chemist LDB, NIDR
Aota S Visiting Associate LDB, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Office of the Director Intramural Research

SECTION

Molecular Structural Biology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fibronectin is a multidomain protein with numerous functions. Our goal is to determine the three dimensional structure of the fragment consisting of the ninth and tenth type III modules. The tenth module contains the RGD cell attachment sequence while the ninth module contains the RGD synergy region. The fragment consisting of the ninth and tenth type III modules has full fibronectin binding activity to the specific integrin receptor, $\alpha 5 \beta 1$. Initial attempts to study the human fragment were frustrated by protein aggregation at concentrations required to carry out NMR experiments. This problem was overcome by expressing the recombinant mouse protein, which yields good quality NMR spectra at concentrations of 0.8mM. Virtually complete signal assignments have been obtained of the two module mouse fragment, and the complete secondary structure of both modules has been determined along with an initial 3D structure of the two module protein. Although we do not yet have a high resolution structure, the available structure does show that the RGD region is on the protein surface and is highly flexible. In addition it is not close to the synergy sequence in the ninth module. Hence the enhanced activity of the module fragment is not due to a direct interaction between the RGD and synergy sequences.

The significance of the project resides in the insight that the structure of the RGD fibronectin fragment will provide about cell attachment and integrin recognition. This information should facilitate the design of more active cell attachment molecules which could improve treatment of diseases resulting from impaired cell adhesion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00645-01 ODIR

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NMR Structure/Function Studies of TGF- β 1

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Torchia DA Chief ODIR, NIDR

Others: Hinck AP IRTA Fellow ODIR, NIDR

COOPERATING UNITS (if any)

NCI, (Roberts A); R&D Systems, (Tsang M, Lucas R).

LAB/BRANCH

Office of the Director Intramural Research

SECTION

Molecular Structural Biology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Among its many functions, TGF- β 1 up- and down-regulates proliferation of HIV infected cells. We have recently completed a determination of the three dimensional structure of TGF- β 1 in solution. The overall structure is similar to the independently determined X-ray structure of TGF- β 1. However, there are significant differences in the local structures of the two proteins, involving residues 69-74 and hydrophobic sidechains of residues in the ranges 24-33 and 88-102. We are currently analyzing the X-ray and NMR data with the goal of deriving a structure based explanation for the differences in the function of the two TGF- β isoforms. The significance of this project arises from the unique, detailed structural information that we are deriving about TGF- β 1 in solution. This information will contribute improved understanding of the structure/function relationships of the family of TGF- β proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DE 00012-33 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infrared and Raman Spectroscopy of Teeth, Bones and Related Synthetic Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B.O. Fowler, Research Chemist, BRB, DIR, NIDR

Others:

COOPERATING UNITS (if any)

ADAHF, NIST, Gaithersburg, MD; NIST, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objective is to determine compositional and structural details of the inorganic phase in teeth and bones. Infrared and Raman spectroscopy, x-ray diffraction and chemical methods are employed in these studies. Methods are devised for the preparation of synthetic calcium apatites having controlled physical properties (crystal size and perfection) and chemical constituents (hydroxide, fluoride, chloride, carbonate, water, acid phosphate and other ions). The vibrational spectra of these apatites and related compounds are assigned and characterized. Isotopically enriched apatite analogs are prepared to facilitate spectral assignments. The spectroscopic assignments and supplemental spectral data (temperature dependence and polarization) are then utilized to establish compositional and structural details of the apatites in question, which include: the type and geometry of constituent ions; the site or number of sites occupied by the ions; orientation of ions; chemical bonding and interactions of ions; and semi-quantitative estimations of the constituents present. The results for these controlled apatite systems are then related to the inorganic phase in calcified tissues. Combined infrared, Raman, and x-ray diffraction methods were used to identify and semi-qualify phases and structural forms of calcium phosphate substances formed upon coating titanium implant surfaces by plasma spraying from hydroxyapatite sources. A large quantity, about one kg, of hydroxyapatite previously prepared from solution, was analyzed in detail by numerous physicochemical methods. This hydroxyapatite was found suitable to be certified as a Standard Reference Material, and it will be available at a future date from NIST.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00074-23 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone and Tooth Matrix Biochemistry and Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L.W. Fisher, Research Chemist, BRB, DIR, NIDR

Others: J.T. Stubbs III, IRTA Fellow, BRB, DIR, NIDR

COOPERATING UNITS (if any)

Sackler School of Medicine, Tel Aviv, Israel; Universita "La Sapienza", Rome, Italy; Dental Research Unit, Hebrew University, Jerusalem, Israel

LAB/BRANCH

Bone Research Branch

SECTION

Protein Chemistry Program, Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For FY 1994 the Protein Biochemistry Program continued its interest in structure-function studies and was involved in one major project and several smaller collaborations. The major project was to identify the specific portions of the bone sialoprotein (BSP) that are involved in the two commonly accepted functions of this sulfated phosphoglycoprotein, cell attachment and the putative ability to nucleate hydroxyapatite crystals. We have identified two cell attachment domains that do not directly involve the cell attachment tripeptide ArgGlyAsp (RGD). The mineral-binding properties appear to be spread over the middle two-thirds of the protein rather than being limited to a small domain. In collaboration with Dr. Dennis Torchia, we have determined that the integrin-binding 59 amino acid RGD domain is a highly flexible coil. Two types of collaborations with scientists outside of NIDR also involve BSP. With Dr. Paulo Bianco in Rome, Italy we are continuing to study the synthesis and secretion of BSP by bone cells and trophoblasts. With two other laboratories in Europe, we are studying the usefulness of BSP as a marker of bone cancers as well as other tumors (breast and prostate cancers) that frequently form mineralized nodules and also metastasize to bone. With Dr. Deutsch in Israel we have continued the collaborative studies on the enamel protein, tuftelin, including human gene sequencing, chromosomal localization, and surveying non sex-linked amelogenesis imperfecta families in Israel. We have completed our collaborations with two groups in involving the human biglycan gene/promoter and its activation status on the X chromosome in human females. A collaboration with a colleague in Israel involving a cDNA clone we discovered that may be a marker of bone stem cells in mice has continued. Finally, our program has sent nearly 400 reagents to over 100 laboratories around the world, over 10% of which were in the dental field.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00088-22 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical, Structural and Morphological Studies on Calcium Phosphates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.D. Eanes, Chief, MCSS, BRB, DIR, NIDR

Others: D. Skrtic, Visiting Associate, BRB, DIR, NIDR

A.W. Hailer, Chemist, BRB, DIR, NIDR

COOPERATING UNITS (if any)

The Hebrew University of Jerusalem, Israel; ADAHF Paffenbarger Research Center, Gaithersburg, MD

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study the physical, chemical, and ultrastructural properties of calcium phosphate salts, and to clarify the kinetic and thermodynamic processes and the interactions with substances of biological interest that uniquely enable calcium phosphate salts to carry out their specialized role in vivo. The properties of calcium phosphate salts are being studied with a variety of ultrastructural and physical-chemical techniques such as electron microscopy, x-ray diffraction, surface area analyses, chromatographic and standard analytical chemistry procedures. The principal endeavor currently being pursued involves artificial lipid vesicles (liposomes) as in vitro models to investigate physico-chemical aspects of matrix vesicle (MV)-mediated calcification in vivo. The latest phase of this endeavor is a study that is examining the effect that organic phosphonates have on mineral development in the liposomal model system. The aim of this study is to better delineate the physicochemical basis for the observed suppressive effects bisphosphonates have on biological calcification. Findings from this study showed that when present in the external suspension solution, the investigated phosphonates did not delay the initial formation of apatitic calcium phosphate salts within the liposomes, nor the penetration of the crystals through the enclosing membranes. However, they variably retarded subsequent growth and proliferation once the crystals were released into the suspending medium. The effectiveness of the phosphonates in inhibiting extraliposomal precipitation is strongly dependent upon their structure, with geminal bisphosphonates having the greatest negative impact. The results suggest that inhibitory binding to crystal growth sites was strongest when the phosphonate molecule contained two phosphonic groups linked to the same carbon atom. The effectiveness of non-geminal bisphosphonates depended upon the presence of electron withdrawing groups, e.g. keto groups, in positions α to the phosphonic groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00379-12 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Bone Matrix Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: M.F. Young, Research Biologist, BRB, DIR, NIDR

Others: J.M. Kerr, Staff Fellow, BRB, DIR, NIDR

K. Ibaraki O'Connor, Visiting Associate, BRB, DIR, NIDR

T. Xu, IRTA Fellow, BRB, DIR, NIDR

M. Donlon, Biological Aide, BRB, DIR, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Molecular Biology Program, Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.00

PROFESSIONAL:

3.83

OTHER:

.17

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The matrix proteins of bones and teeth play key roles in the structure and function of these tissues. Our objective is to study the structure and function of these macromolecules and to understand the regulation of their expression. The primary structures of bone and tooth matrix proteins have been studied by constructing recombinant cDNA libraries from bone or ameloblast cell mRNA. cDNAs encoding several bone and tooth matrix proteins were isolated. The clones and antibodies were used to determine the primary structure and mode of expression of the genes in cultured cells and intact tissue. These studies showed the matrix proteins had distinct spatial and temporal patterns of expression during bone cell differentiation both in vitro and in vivo. Using the cDNAs as probes we have isolated genomic DNA and analyzed the control of certain of these genes at the nuclear level. The human biglycan promoter was shown to contain a functional G rich region between -262 and -218 that bound to a novel 25 kDa nuclear factor present in cultured bone cells. The controlling elements of the BSP gene were also characterized and indicated that the basal promoter was regulated by a transcription factor known as YY1 (yin yang 1). Studies are underway using transgenic mice to identify the function of the matrix proteins and the elements that regulate their expression during development and aging in vivo.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 DE 00380-12 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Bone Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: P. Gehron Robey, Chief, BRB, NIDR

Others: W.J. Grzesik, Visiting Associate, BRB, NIDR

C. Crescioli, Visiting Fellow, BRB, NIDR

S. Kuznetsov, Visiting Associate, BRB, NIDR

D.W. Rowe, IPA, BRB, NIDR

A. Majolagbe, Biological Aide, BRB, NIDR

S. Satomura, Visiting Fellow, BRB, NIDR D. Benayahu, Visiting Fellow, BRB, NIDR

COOPERATING UNITS (if any)

Department of Biopathology, Universita "La Sapienza", Rome, Italy; Department of Cell Biology and Histology, Tel Aviv University, Tel Aviv, Israel

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.07

PROFESSIONAL:

4.07

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary goals of the Cellular Biochemistry Group are to determine the composition and functional features of the supramolecular complex of proteins that calcifies, and how cells regulate this process. Towards these aims, cell cultures that support and form mineralized tissues were established for biochemical analysis, and for studies at the genomic level in collaboration with Drs. Marian F. Young and Larry W. Fisher. Studies were initiated to characterize the biosynthetic products of a cell line BBE (bovine bone endothelial cells) since the vasculature has been shown to influence bone metabolism. It was found that these cells synthesize fibronectin, type I collagen, osteonectin and thrombospondin, and induce the preosteoclastic cell line, FLG 29.1, to attach and multinucleate. Further characterization of FLG 29.1 also indicated that they synthesize bone sialoprotein, as has been observed in bona fide osteoclasts by in situ hybridization and immunohistochemistry. Continued analysis of cell-matrix interactions indicate that the two small proteoglycans found in bone, biglycan and decorin, inhibit bone cell attachment to some but not all RGD-containing proteins, suggesting that osteoblastic cells can modulate their attachment to the surrounding environment by the production of such inhibitory molecules. Cell attachment to bone matrix proteins was also found to depend on the phenotypic traits and maturational stage of cloned mouse marrow stromal fibroblasts. Lastly, studies utilizing bone-forming cultures from patients with different forms of osteogenesis imperfecta indicates that there is a major change in the stoichiometry of extracellular matrix proteins, irrespective of the detectability of a collagen mutation. While synthesis of versican, biglycan, decorin and osteonectin are decreased, thrombospondin and fibronectin are increased. These changes may contribute significantly to cell-matrix interactions and may play a role in the pathophysiology of this brittle bone disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DE 00548-04 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Cellular Metabolism of Proteoglycans Using Brefeldin A

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Masaki Yanagishita, Visiting Scientist, BRB, DIR, NIDR

Others: Anthony Calabro, Staff Fellow, BRB, DIR, NIDR

Vincent Hascall, Guest Researcher, BRB, DIR, NIDR

COOPERATING UNITS (if any)

Department of Biochemistry, University of Tromso, Norway

LAB/BRANCH

Bone Research Branch

SECTION

Glycobiology Program, Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.10

PROFESSIONAL:

1.10

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The glycosaminoglycan components of proteoglycans are biosynthesized and modified in the golgi apparatus by highly organized carbohydrate transfer enzymes and sulfotransferases. The purpose of this project is to investigate the functional organization and subcellular localization of these enzyme complexes. Brefeldin A (BFA) is a chemical which specifically blocks anterograde protein transport within the golgi apparatus. It was used to disrupt the normal biosynthetic processes for adding glycosaminoglycan chains onto proteoglycans. We examined the effects of BFA on the synthesis of hyaluronan (HA) and aggrecan, two of the major extracellular matrix molecules, in rat chondrosarcoma cells. Biosynthesis of chondroitin sulfate (CS) associated with aggrecan was rapidly inhibited to >1% of the control, while that of HA continued at the normal level. This result was consistent with the current model that the biosynthesis of CS requires the transport of the core protein through the Golgi apparatus, while that of HA occurs at the plasma membrane and therefore is independent of the vesicular transport. When ovarian granulosa cells were treated with BFA, dermatan sulfate proteoglycan synthesis was abolished whereas heparan sulfate proteoglycan synthesis was only partially inhibited, suggesting that dermatan sulfate and heparan sulfate assembly on proteoglycans occurs in different subcellular compartments in these cells. The finding that only normal heparan sulfate protein core proteins were substituted with heparan sulfate chains in the presence of the drug indicated that glycosylation enzymes are highly specific to core proteins. Topics of present interest include elucidation of core protein structure which determines highly specific glycosylation enzymes and the effect of brefeldin A on endocytotic mechanism involving cell surface proteoglycans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00549-04 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Cell Surface Heparan Sulfate Proteoglycans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Masaki Yanagishita, Visiting Scientist, BRB, DIR, NIDR

Others: Duncan Hiscock, Visiting Associate, BRB, DIR, NIDR

Chee Keng Ng, Visiting Fellow, BRB, DIR, NIDR

COOPERATING UNITS (if any)

Division of Cytokine Biology, CBER, FDA

LAB/BRANCH

Bone Research Branch

SECTION

Glycobiology Program, Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.35

PROFESSIONAL:

2.35

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cell surface heparan sulfate proteoglycans are widely distributed throughout animal tissues, and are involved in critical cell-functions such as cell-cell and cell-extracellular matrix interactions. Their interaction with a variety of molecules including growth factors, viruses, and extracellular matrix proteins, have important biological functions. The purpose of this project is to study the metabolism of cell surface heparan sulfate proteoglycans with focus on mechanisms involved in their endocytosis and subsequent intracellular processing. We have studied intracellular localization of heparan sulfate proteoglycan (HSPG) and dermatan sulfate (DS) PGs in an osteoblastic cell line (UMR 106), oral epithelial cells and retroocular fibroblasts using metabolic radiolabeling experiments in combination with subcellular fractionation techniques and examination with electron microscopy. Results indicated that a large proportion of HSPGs formerly identified in nuclear fractions are contamination from plasma membranes and that the small DSPG (probably biglycan and/or decorin) which associates with the cell surface may well traffic through the nucleus in these cells. We also studied biosynthesis of bone sialoprotein (BSP) by a human osteoclastic cell line (FLG 29.1) during its differentiation induced by TPA using metabolic radiolabeling experiments. Topics of present interest include: (1) the study of mechanisms regulating expression of cell surface HS proteoglycans in various cell types, and (2) interactions of cell surface proteoglycans with growth factors, chemokines and viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00552-04 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Differentiation Factors in Cartilage and Bone Formation and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Frank P. Luyten, Visiting Scientist, BRB, DIR, NIDR

Others: John T. Thomas, Visiting Associate, BRB, DIR, NIDR

Keming Lin, Visiting Fellow, BRB, DIR, NIDR

Maithily Nandedkar, Special Volunteer, BRB, DIR, NIDR

COOPERATING UNITS (if any)

School of Medicine, Zagreb, Croatia; Creative BioMolecules, Hopkinton, MA; Center for Biologics Evaluation and Research, FDA; Laboratory of Molecular Biology, NIAID

LAB/BRANCH

Bone Research Branch

SECTION

Developmental Biology Project, Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.72

PROFESSIONAL:

2.0

OTHER:

.72

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to study the cartilage and bone inducing factors and to define their role in embryogenesis and in postnatal life, both in tissue formation and in disease. As tissue regeneration recapitulates the developmental sequence of embryonic tissue formation, it is conceivable that understanding the mechanisms of action of the soluble differentiation factors is a key step towards biologically controlled regeneration of skeletal tissues. This will have a significant impact on the treatment of congenital and/or acquired skeletal diseases such as large bone defects, impaired fracture healing, osteoarthritis, osteoporosis and periodontitis. This project focuses on the further characterization of cartilage and bone inducing molecules, members of the TGF-B superfamily, and their biological activities. Using molecular probes, we are studying their respective contributions to cartilage and endochondral and membranous bone formation. Immunohistochemical localization and in situ hybridization of cartilage and bone inducing proteins, as well as studies in vitro, indicate the selective contribution of these molecules to initiation, enhancement, maintenance and maturation of the chondrocytic and osteoblastic phenotypes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DE 00554-04 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physicochemical Studies on Calcium Phosphate Cements

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.D.Eanes, Chief, MCSS, BRB, DIR, NIDR

Others:

COOPERATING UNITS (if any)

Dental and Medical Materials Group, Polymers Division, NIST, Gaithersburg, MD; ADAHF
Paffenbarger Research Center, NIST, Gaithersburg, MD; Tokushima University, Japan

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.1

PROFESSIONAL:

.1

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Self-setting calcium phosphate cements (CPC) are promising materials which have a variety of possible medical and dental applications. *In situ* setting and biocompatibility properties make CPCs potentially useful as endodontic filling materials, as implants for bony defects, and as a binder for other implant materials. CPCs are formed by moistening biphasic mixtures of calcium phosphate salts, usually anhydrous dicalcium phosphate (DCPA) and tetracalcium phosphate (TTCP), with limited amounts of water. Although relatively simple materials in composition, other chemical as well as physical properties, e.g. setting times, porosity and strength, are dependent in a complex manner upon a number of poorly understood parameters associated with the chemistry of the setting process. Particularly relevant are the solution parameters important in establishing the crystalline texture (i.e., size, shape, and aggregation properties) of the apatitic product formed upon completion of the DCPA/TTCP conversion, since the texture of this phase is a major determinant of the mechanical behavior of these cements. Thus, studies of solution influences on apatite crystal growth may prove useful in formulating cements with improved mechanical properties.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 DE 00574-03 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amorphous Calcium Phosphate as an Inorganic Component in Dental Materials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Skrtic Visiting Associate, BRB, DIR, NIDR

Others: E.D. Eanes, Chief, MCSS, BRB, DIR, NIDR

A.W. Hailer, Chemist, BRB, DIR, NIDR

COOPERATING UNITS (if any)

Dental and Medical Materials Group, Polymers Division, NIST

LAB/BRANCH

Bone Research Branch

SECTION

Mineral Chemistry and Structure

INSTITUTE AND LOCATION

NIDR, DIR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

0.9

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, amorphous calcium phosphate (ACP), an important intermediate in the formation of apatite, is being investigated for possible use as a dental material. When either used alone, or in combination with other dental materials, especially polymeric resins, ACP has a wide range of possible applications such as in restorative composites, cavity liners and bases, luting and pulp capping agents, prophylactic and endodontic sealants, and as a component in periodontic packs and impression pastes. It has a number of potential advantages over other calcium phosphates for these purposes. As a dental cement, its advantage over current biphasic systems (e.g., dicalcium/tetracalcium phosphate mixtures) is its simpler, single solid phase formulation. When included as a component in appropriate resin-based composites, sealants and adhesives, ACP may be useful as a remineralization agent as well as a vehicle for sustained, controlled release of inorganic anticaries ions such as fluoride. In this regard, chemical studies on various ACP-resin formulations indicate that ACP-embedded, methacrylate resins release calcium and phosphate ions at levels that exceed the thermodynamic minimum necessary for remineralizing damaged tooth surfaces. Currently, in vitro studies on bovine teeth are being carried out to evaluate the suitability of ACP-resin composites as remineralizing dental sealants. Results to date indicate that in artificial saliva-like test solutions, significantly higher levels of remineralization occur in tooth lesions coated with ACP-containing resins compared to uncoated lesions or those coated with silica- or apatite-containing resins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00611-02 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Bone and Mineral Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kazushige Sakaguchi, Visiting Scientist, BRB, NIDR

Others: Masaki Yanagishita, Visiting Scientist, BRB, NIDR

Koichi Minami, Guest Researcher, BRB, NIDR

COOPERATING UNITS (if any)

Endocrinology and Reproduction Research Branch, NICHD; Department of Clinical Physiopathology, University of Florence; Laboratory of Cellular and Molecular Biology, NCI

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology Section, Glycobiology Program

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.40

PROFESSIONAL:

1.40

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The parathyroid gland is the major endocrine organ which regulates extracellular calcium by secreting parathyroid hormone (PTH). Extracellular calcium is not only vital to fundamental cellular functions, but also plays pivotal roles in the metabolism of skeletal tissues and teeth. The feed-back mechanism of calcium regulation by PTH involves sensing of extracellular calcium concentration by parathyroid cells and mobilization of calcium by PTH at peripheral tissues. Among the peripheral tissues responsive to PTH, cells in an osteoblast lineage appear to be most important in inducing bone resorption by osteoclast and in regulating extracellular calcium concentration. The main purpose of this program is to study (1) how parathyroid cells sense extracellular calcium concentration, (2) how this signal regulates parathyroid cell functions, including cell growth and hormone secretion, and (3) how functions of osteoblasts and osteoclasts are regulated by PTH, cytokines and growth factors.

We have established a unique parathyroid cell line which retains its physiological functions in culture. Parathyroid cell growth regulation by extracellular calcium has been demonstrated by us to be highly dependent on an autocrine system involving acidic fibroblast growth factor (aFGF) and its cell surface receptor (aFGF-R). The mechanism of PTH secretory regulation by calcium is currently not well understood. The calcium-sensing receptor, a G-protein coupled receptor, on the parathyroid cell surface appears to play a major role, but there is evidence that other factors modulate the regulation mediated by the calcium-sensing receptor. We have previously reported that endothelin 1 (ET-1) and its receptor (ETA receptor) form an autocrine loop in parathyroid cells, and that this autocrine loop is regulated by extracellular calcium concentration. In another aspect of mineral metabolism, to understand the regulatory mechanisms of osteoblasts by external stimuli, it is important to know intracellular signal transduction pathways unique to them.

The topics of current interest are to study effects of extracellular calcium on the expression and post-translational modification of aFGF and aFGF-R by parathyroid cells, to understand the regulatory mechanisms of PTH secretion by the ET-1/ETA receptor autocrine loop, and to elucidate the signal transduction pathways unique to the cells in an osteoblast lineage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00643-01 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Cell Surface Heparan Sulfate Proteoglycans in HIV Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Masaki Yanagishita, Visiting Scientist, BRB, DIR, NIDR

Others: Duncan Hiscock, Visiting Associate, BRB, DIR, NIDR

COOPERATING UNITS (if any)

Division of Cytokine Biology, CBER, FDA; Department of Biochemistry, University of Tromso, Norway

LAB/BRANCH

Bone Research Branch

SECTION

Glycobiology Program, Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.40

PROFESSIONAL:

.40

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Elucidation of the early molecular events associated with human immunodeficiency virus type 1 (HIV-1) infection is of major importance to AIDS therapeutics and vaccine development. Although cell surface CD4 is the primary receptor for HIV-1, evidence suggests that other cell surface molecules, either independently or in association with CD4, may participate in virus binding and entry. We have shown that cell surface heparan sulfate (HS) proteoglycans participate in the infection of CD4+ T-cell lines. Removal of HS from the cell surface by specific enzyme digestion or the reduction of the sulfation of HS chains by a metabolic competitor reduced virus binding and infection. The data also supported that both HS and CD4 receptor participate in either initial virus attachment or postbinding events, and that the V3 domain of an HIV-1 envelope protein, gp120, interacts with cell surface HS proteoglycans. Topics of current interest include (1) analysis of molecular interactions between HIV-1 envelope proteins and cell surface HS proteoglycans, and (2) biochemical characterization of HS proteoglycans in HIV-1 target T-lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00646-01 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Frizzled-like Proteins in Skeletal Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Frank P. Luyten, Visiting Scientist, BRB, DIR, NIDR

Others: John T. Thomas, Visiting Associate, BRB, DIR, NIDR

Keming Lin, Visiting Fellow, BRB, DIR, NIDR

Maithily Nandedkar, Special Volunteer, BRB, DIR, NIDR

COOPERATING UNITS (if any)

School of Medicine, Zagreb, Croatia; Center for Biologics Evaluation and Research, FDA; Laboratory of Molecular Biology, NIAID

LAB/BRANCH

Bone Research Branch

SECTION

Developmental Biology Project, Skeletal Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.11

PROFESSIONAL:

1.0

OTHER:

.11

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this project is to study the role of a novel family of frizzled-like genes in skeletal development, regeneration and disease.

The discovery of a novel gene designated *frzb* in our laboratory and the dynamics of its expression pattern predominantly restricted to the developing skeleton during human embryonic development, presents us with a unique opportunity to study a complete new class of genes intimately involved in skeletal morphogenesis. *Frzb* is related to the *Drosophila* polarity gene *frizzled*, which encodes an integral membrane protein. This locus is required for the cellular response to a tissue polarity signal. Strong *frizzled* mutations are associated with random orientation of wing hairs in the fruit fly.

The study of this group of polarity determining genes should allow us to further define the cell surface proteins/membrane receptors, their ligands and signal transduction pathways directly involved in the patterning process during the development of skeletal structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 DE 00649-01 BRB

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bone Regeneration Using Marrow Stromal Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. Gehron Robey, Chief, BRB, DIR, NIDR

Others: S. Kuznetsov, Visiting Associate, BRB, DIR, NIDR

M. Mankani, Special Volunteer, BRB, DIR, NIDR

A. Aaron, Special Volunteer, BRB, DIR, NIDR

P. Krebsbach, Senior Staff Fellow, LDB, DIR, NIDR

J. Brahim, Chief Maxillofacial Surgeon, CIPCB, DIR, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Bone Research Branch

SECTION

Skeletal Biology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.47

PROFESSIONAL:

2.47

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

It is now well established that bone marrow contains a population of cells, marrow stromal fibroblasts (MSFs, also known as CFU-f), that have the potential to form bone/cartilage, adipose and hematopoiesis support tissue, depending on the microenvironment and systemic and local growth factors that they are exposed to. The ability of human marrow stromal cells, derived from bone aspirates from patients recruited under protocol 94-D-0188 to form new bone when implanted into immunosuppressed animals was investigated. It was determined that 1) in vitro expansion of the osteogenic cells in the population of human marrow cells requires the continuous presence of dexamethasone and ascorbate-2-phosphate and that 2) small particles of tricalcium phosphate - hydroxyapatite ceramic with attached human MSFs held together by a fibrin clot supports exuberant new bone formation upon implantation into immunosuppressed mice and 3) the new bone formed appears to be lamellar in character indicating a bypass of the woven bone stage usually observed in de novo bone formation. These results indicate efficacy of this system for bone augmentation and will serve as the basis for future protocols designed to perform these procedures in human patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00212-19 CIPC

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Taste and its Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Weiffenbach, James

Research Psychologist

CIPC NIDR

Fox, Philip C.

Dental Officer

CIPC NIDR

COOPERATING UNITS (if any)

LSB, NIH; Audie L Murphy Memorial Veterans Hospital, San Antonio, Texas; Yale University School of Medicine New Haven, Connecticut; University of Washington, Seattle, Washington; Francis Scott Medical Center, Baltimore, Maryland

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS

1.0

PROFESSIONAL

1.0

OTHER

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project seeks to elucidate the mechanisms by which oral perceptual experience is generated. Since objective measurement of the various aspects of oral experience is fundamental to this effort, the selection and refinement of appropriate psychophysical methods is a primary and continuing project concern. Currently, the routine assessment of taste is carried out using aqueous solutions representing each of the four basic tastes. Measures include both (detection) thresholds and judgments of intensity for taste stimuli at higher, more commonly encountered levels of strength. Assessments of sensitivity to localized taste and touch on the tongue and to variation in the temperature or viscosity of an oral bolus are also available. Olfactory function is routinely assessed by a standardized test of odor identification. These methods are used to study oral sensory changes that may occur with oral or systemic disease and its treatment, with salivary gland dysfunction, with aging or in association with an isolated oral or taste complaint. Such studies can provide insight in the sensory mechanisms that normally provide for the perception of the complex oral stimuli encountered in everyday life but may, in other circumstances, produce distressing and debilitating oral symptoms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00332-14 CIPC

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Oral Medicine Program: Clinical Investigations and Case Reports

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Kohn, William G.	Dep Clinical Director	CIPC	NIDR
Adesanya, Margo	Clinical Associate	CIPC	NIDR
Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
Baum, Bruce J	Clin. Director/Chief	CIPC	NIDR
Fox, Philip	Chief, CIS	CIPC	NIDR
Grisius, Margaret	Clinical Associate	CIPC	NIDR
McCarthy, George M.	Senior Staff Dentist	CIPC	NIDR
Meehan, Sean	Clinical Associate	CIPC	NIDR

COOPERATING UNITS (if any)

Lab. of Clin. Sci., NIMH; Pediatrics Branch, NCI; Inter-Inst. Genet. Prog., CC; Clinical Pathology Lab, CC; Clin. Genet. Sect., NCI; Dermatology Branch, NCI

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

1.33

PROFESSIONAL:

.8

OTHER:

.53

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Clinical case studies of unusual interest and patient-related research are conducted on a variety of oral and dentally-related subjects within the context of our Oral Medicine training program. The CIPCB sponsors an Oral Medicine Fellowship that provides hospital-trained dentists (1-2/year), interested in a career in academic oral medicine and dental research, with a 3-year, high-quality clinical and basic science research experience. Research techniques range from chart and literature reviews, to the direct evaluation and utilization of advanced diagnostic and therapeutic regimens. Interesting problems of oral pathology, with or without other medical complications, are often seen in the consult clinic. The study and publication of such cases provide valuable information for the practicing dental clinician as well as a rich training experience for the Oral Medicine fellows. The NIDR clinic is a Dental/Oral Medicine consult clinic for all the NIH institutes. Fellows participate in the diagnosis and treatment of medically-compromised patients referred to the clinic and are encouraged to participate in studies of the oral manifestations of systemic disorders and of the oral complications of medical therapy. Particular emphasis is placed on oral mucosal disorders, with special attention to potential saliva/mucosal interactions. The biological/pathophysiologic implications of clinical conditions are discussed and framed into scientific questions with the help of a staff mentor. Collaborations with investigators at other institutes, utilizing the unique patient populations present at NIH, are encouraged.

Professional Personnel, continued

O'Connell, Anne	Senior Staff Dentist	CIPC	NIDR
Tessler, Sara	Clinical Associate	CIPC	NIDR
Wu, Ava	Senior Staff Dentist	CIPC	NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00336-14 CIPC

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Salivary Gland Secretion Mechanisms During Normal and Altered Functional States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Baum, B.J.	Clinical Dir/Chief	CIPC	NIDR
Braddon, V.	Staff Fellow	CIPC	NIDR
Adesanya, M.	Clinical Associate	CIPC	NIDR
Delporte, C.	Visiting Fellow	CIPC	NIDR
He, X.	Visiting Associate	CIPC	NIDR
Lazowski, K.	Visiting Associate	CIPC	NIDR
Lillibridge, D.	Staff Fellow	CIPC	NIDR
O'Connell, B.	Sr. Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

CP, CC, NIH; Oral Pathology, VAMC, Washington, DC; Dept. of Oral Biology, Boston Univ.; Dept. of Biological Chemistry, Johns Hopkins Univ.; Dept. of Dental Resch., Univ. of Rochester, Dept. of Biochemistry & Mol. Biology, Med. Univ. of South Carolina, Charleston; Dept. of Cell Biology, Aarhus Univ., Denmark

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Gene Transfer Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS

6.12

PROFESSIONAL

4.87

OTHER

1.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The health of the oral cavity is maintained by salivary secretions. The principal function of salivary glands is to produce these complex fluids. We have utilized a variety of tools to understand saliva formation and the pathologic processes that alter normal secretory events. During this reporting period, we have concentrated our efforts on an important issue relevant to the development of clinically-applicable gene transfer vectors for use in salivary glands: the relatively short time of transgene expression which we have previously reported occurs with this tissue. We have approached this in three general ways: (i) to understand, and attempt to control, the inflammatory response which occurs following adenovirus administration; (ii) to re-engineer the recombinant vectors to render them more potent, thus allowing lower doses of virus to be used in vivo; and (iii) to evaluate further the use of an adeno-associated viral vector as a gene transfer vehicle (since this virus is able to integrate into the host cell DNA) in place of adenoviral vectors. A related, but separate, major effort has been directed at defining salivary cell type (acinar, ductal)-specific promoters. Their utilization in place of the viral promoters currently employed should enhance considerably vector safety and the stability of gene expression. Further, this work of itself makes fundamental contributions to the biology of salivary glands. Additionally, we have continued the studies that we began last year of the topological "mapping" of key plasma membrane transport proteins, and associated protein routing signals, in salivary cells.

Professional Personnel, continued

Park, C.

Visiting Fellow

CIPC

NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00337-14 CIPC

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Physiological Processes: Normal Function and Disease Perturbation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Fox, Philip C.	Dental Officer/Chief, CIS	CIPC	NIDR
Ambudkar, Indu	Biologist/Chief, SPS	CIPC	NIDR
Baum, Bruce J.	Clinical Dir/Chief, CIPC	CIPC	NIDR
Gannot, Gallya	Special Volunteer	CIPC	NIDR
Grisius, Margaret M.	Clinical Associate	CIPC	NIDR
Macynski, Alice A.	Research Nurse	CIPC	NIDR
Park, C	Visiting Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

ARB, NIAMS; RM, CC; DDEM, NIDDK; DVP, CBER, FDA; Baylor Univ., Dallas, TX; Howard Univ., Washington, DC; Univ. of Missouri, Kansas City, Mo; Univ. of Liverpool, England; Eastman Dental Institute., London, England

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS.

6.55

PROFESSIONAL:

4.55

OTHER:

2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines the function of the salivary glands and other oral tissues in individuals with alterations of normal oral function due to disease or therapeutic procedures. Entry into all studies is through the Dry Mouth Clinic. Utilizing outpatient and inpatient services, specific evaluative and diagnostic approaches establish the cause and extent of salivary gland dysfunction in the "dry mouth" patient. The focus of clinical studies for many years has been primary Sjögren's syndrome, a systemic autoimmune exocrinopathy with major manifestations of chronic, progressive salivary and lacrimal gland dysfunction, and irradiation-induced salivary gland dysfunction. Oral and secretory effects of other selected systemic diseases also are evaluated. An ongoing therapeutic protocol is evaluating the effectiveness of the combination of the anti-inflammatory drug hydroxychloroquine and the parasympathomimetic secretagogue pilocarpine HCl for treatment of salivary, lacrimal, and serological disease in primary Sjögren's syndrome patients. Clinical research studies are i) seeking better salivary and serologic markers of exocrine disease activity in this disorder; ii) defining the expression of adhesion molecules and cytokine receptors in the salivary glands of Sjögren's syndrome patients and normal controls; and iii) evaluating disease progression over time. Laboratory studies focus on the immunopathological mechanisms of salivary dysfunction found in Sjögren's syndrome. Cytokine expression in salivary glands and the effects of cytokines and other immune mediators on a cultured human salivary ductal cell line have been investigated.

Professional Personnel, continued

Sun, Di
Weiffenbach, James M.
Wu, Ava J

IRTA Fellow
Research Psychologist
Senior Staff Dentist

CIPC NIDR
CIPC NIDR
CIPC NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00412-10 CIPC

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Endosseous Titanium Implants in Edentulous and Ectodermal Dysplasia Patients.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Brahim, Jaime S.	Senior Staff Dentist	CIPC	NIDR
Kohn, William G.	Chief, PCCSS	CIPC	NIDR
McCarthy, George R.	Senior Staff Dentist	CIPC	NIDR
O'Connell, Brian	Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

Rehabilitation Medicine Department CC; Nutrition Department, CC; Surgical Services Department, CC; Commissioned Officers Dental Clinic, CC; Nursing Department, CC.

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Patient Care and Clinical Studies Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:

3.53

PROFESSIONAL:

1.35

OTHER:

2.18

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project examines the use of endosseous, root form, dental implants in completely edentulous patients, pre-adolescent, adolescent, and adult patients with ectodermal dysplasia (ED), and in adult patients (over 18 years) that require the replacement of single teeth. The implants are utilized to support a fixed dental prosthesis. Removable dentures are considered a significant handicap related to mastication, speech, esthetics, continued reduction of the residual ridges of the mandible and maxillae, and body self-image. Individuals affected by ectodermal dysplasia can have multiple congenitally absent teeth. Consequently, the alveolar bone fails to achieve normal height and volume, as it is dependent upon the development and eruption of the teeth. A lack of alveolar bone not only makes removable denture wear extremely difficult, but also the placement of endosseous implants problematic and potentially less successful. These studies attempt to determine if: (1) endosseous dental implants can be used successfully in non-ED edentulous adult patients and in pre-adolescent, adolescent, and adult ED patients with multiple congenitally missing teeth; and (2) coating a titanium alloy implant with hydroxyapatite improves its success when used to replace single missing teeth. Additionally, we will assess over the duration of this 5-year study if an implant-supported fixed denture significantly affects an individual's loss of vertical dimension of occlusion, satisfaction with treatment, food choice and nutrition, perception of ease/difficulty of chewing selected foods, and body self-image when compared to treatment with a conventional removable denture. Information concerning the relationship of personality to body image and the ability to adapt to oral prostheses of various types will also be assessed. Finally, the project will evaluate the effects, if any, that mandibular endosseous dental implants have on the growth and development of the craniofacial complex of pre-adolescent (7-11 year-old) patients with ED and significant hypodontia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00415-10 CIPC

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ion Transport and Fluid Secretion in Salivary Glands

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Turner, R. J.	Chief/MBS	CIPC	NIDR
Moore, M.L.	Staff Fellow	CIPC	NIDR
Evans, R.L.	Visiting Fellow	CIPC	NIDR
Kurihara, K.	Visiting Fellow	CIPC	NIDR
Yamane, J.	Guest Researcher	CIPC	NIDR
Tessler, S.	Clinical Associate	CIPC	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Membrane Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

4.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Saliva is the principal protective agent for the mouth and thus is of primary importance to oral health maintenance. Perturbations of salivary secretion mechanisms can consequently lead to serious oral health problems. The objective of this project is to study the membrane and cellular processes which underlie the phenomenon of salivary fluid secretion and thus to contribute to our understanding of the fluid secretory process in normal and diseased states. Because similar secretory mechanisms are thought to be common to a number of other exocrine glands, this information should be of rather broad applicability and interest. During the present reporting period our specific areas of focus were the following. (1) Studies of the regulation of the rat parotid acinar Na-K-2Cl cotransporter by muscarinic stimulation were continued. (2) The properties of the anion binding sites on the parotid Na-K-2Cl cotransporter were characterized. (3) Cloning and sequencing of the rat parotid Na-K-2Cl cotransporter was continued and functional expression studies were begun. (4) The effects of IP3 on calcium release from intracellular stores were studied in permeabilized HSY cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00438-09 CIPC

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Molecular Mechanisms Regulating Ca^{2+} Flux in Salivary Glands

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

Ambudkar, Indu	Chief, Secretary Physiology Section	CIPC	NIDR
Baum, Bruce J.	Clin Dir/ Chief, CIPCB	CIPC	NIDR
Lockwich, Timothy	Senior Staff Fellow	CIPC	NIDR
Sakai, Takayuki	Visiting Fellow	CIPC	NIDR
Chauthaiwale, Jyothi	Visiting Fellow	CIPC	NIDR
Meehan, Sean	Clinical Associate	CIPC	NIDR
O'Connell, Anne	Staff Fellow	CIPC	NIDR

COOPERATING UNITS (if any)

Division of Nephrology, Dept. Medicine, Johns Hopkins Univ. School of Medicine; LCB, NIDDK; LCMB,

LAB/BRANCH

Clinical Investigations and Patient Care Branch

SECTION

Secretary Physiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS.

5.3

PROFESSIONAL:

5.25

OTHER

.05

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided)

This project is directed towards understanding the processes which regulate cytosolic $[Ca]$ in salivary gland cells. In these cells, Ca entry which is critical for prolonged fluid secretion in this gland, is regulated by a "Ca release-activated Ca entry"-type of mechanism, found in a number of non-excitabile cells. This largely uncharacterized mechanism appears to be stimulated by the depletion of Ca in the intracellular Ca store(s). In this reporting period we have demonstrated that Ca influx in rat parotid and HSG cells (derived from the human submandibular gland) is regulated by (i) Ser/Thr phosphorylation (inhibition) and dephosphorylation (stimulation). We had described previously that internal Ca pool-depleted parotid acinar cells have two Ca influx components with high and low affinities for Ca , $K_d=65\mu M$ and $K_d=3.3mM$, respectively. In this reporting period we have shown that the high affinity component is more sensitive to inhibition by depolarization and Zn , while both components are inhibited by micronazole and carbodiimide. Further, we have examined the kinetics of Ca influx into isolated basolateral membrane vesicles (BLMV) at a lower temperature ($30^\circ C$) and have demonstrated the presence of two Ca influx components with K_d similar to that found in intact cells ($108\mu M$ and $31.5mM$). In the last reporting period we had described reconstitution of the high affinity Ca^{2+} influx component from BLMV into artificial lipid vesicles, by using a detergent, octylglucoside, dilution method. By using lectin chromatography and the same reconstitution procedure, we have now partially purified this Ca influx component. Continuing our recent studies on the effects of $IFN-\gamma$ and $TNF-\alpha$ on the proliferatin and Ca signalling mechanisms in HSG cells, we have now shown that there is a decrease in the SERCA 2 type of Ca pumps in $IFN-\gamma$ -treated cells. This is a novel observation and likely accounts for the decreased Ca content of internal Ca store(s) in $IFN-\gamma$ -treated HSG cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00311-15 LCDO

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Essential Cellular Function of Hypusine in eIF-5A

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Park, Myung Hee Research Chemist LCDO, NIDR

Others: Joe, Young Ae Visiting Fellow LCDO, NIDR
Lee, Young Bok Visiting Fellow LCDO, NIDR

COOPERATING UNITS (if any)

Dr. Philip Coffino, University of California, San Francisco, CA.
Dr. John W.B. Hershey, University of California, Davis, CA

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.18

PROFESSIONAL:

1.18

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Eukaryotic protein translation initiation factor 5A (eIF-5A) contains one residue of hypusine and appears to be the only cellular protein with this unique amino acid. Hypusine is produced post-translationally by transfer of the butylamine portion of the polyamine spermidine to a lysyl residue in the eIF-5A precursor to form deoxyhypusine followed by hydroxylation to form hypusine.

The precise physiological role of the hypusine-containing protein eIF-5A is yet unknown. However, it is well established that hypusine is vital for eukaryotic cell proliferation. The strict conservation of the sequence of twelve amino acids surrounding the hypusine residue further emphasizes the importance of this residue. We investigated the basis for the specificity of hypusine synthesis with respect to the substrate protein using fragments of eIF-5A precursor protein as substrates for deoxyhypusine synthase, the first enzyme in hypusine biosynthesis. These were generated either by specific endoproteinases or by recombinant deletion subcloning. The results define the minimum domain of the eIF-5A precursor protein required for deoxyhypusine synthesis as Phe³⁰-Asp⁸⁰ and provide insight into the molecular interaction between eIF-5A precursor and deoxyhypusine synthase.

In an effort to identify cellular proteins with which eIF-5A interacts to exert its biological activity, we have originated an affinity method for their isolation. Polyhistidine-tagged human eIF-5A precursor protein (His-tag-ec-eIF-5A) was produced by utilizing pET-24b vector. The deoxyhypusine-containing form was prepared by modification of His-tag-ec-eIF-5A in the deoxyhypusine synthase reaction. The his-tagged proteins bound to a Ni (II)-NTA-agarose column are being used as an affinity matrix to isolate and identify cellular proteins that specifically interact with eIF-5A.

In a recent study from another laboratory, eIF-5A was reported to be a host cellular factor required for Rev function in the replication of HIV-1. We are currently investigating the role of hypusine modification in the interaction of eIF-5A and Rev and the possible intervention of HIV-1 replication through inhibition of hypusine synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER Z01DE00433-09 LCDO
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developing Synthetic Cell Targeting System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Robey, Frank A.	Chief, PIU LCDO, NIDR
	Ivanov, Boris	Visiting Fellow LCDO, NIDR
COOPERATING UNITS (if any) Niels H.H. Heegaard, States Serum Institute, Copenhagen, Denmark; Ivan Stengle, McGill University Montreal Canada; Richard Timmons, University of Texas, Arlington, TX; Gabri van der Plum, University Hospital, Leiden, The Netherlands		
LAB/BRANCH Laboratory of Cellular Development and Oncology		
SECTION Peptide and Immunochemistry Unit		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.25	PROFESSIONAL: 1.25	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The goals of the work performed for this project are the syntheses and uses of new biologically active materials that specifically interact with cell surface receptors and to use the newly designed materials for a variety of functions that include drug and gene delivery, vaccines and substrata for implant materials. A large portion of the work effort lies in the design of new synthetic methods and these are based on the haloacetyl chemistry developed over the past 9 years in this Unit. With these new biomaterials, new assay systems have to be developed and, as such, much of the work effort for this project includes new methods development. However, the primary focus of the work is not in the methods development but in the uses of the new materials in defined biological systems. In addition, much of the work effort emphasizes structure-function relationships. To make a targeted anti-viral vaccine, the knowledge of what components on the virus' surface bind to the cells receptor is necessary. When that information is not available as is the case for gp120 of HIV-1, we construct materials based on theoretical considerations. We have focussed on three proteins: serum amyloid P component (SAP), bone sialoprotein (BSP) and CD4, the cell surface receptor for HIV-1. All three proteins are known to be involved in the interactions of extracellular components having nonspecific characteristics. SAP is known to be involved in binding sulfated polysaccharides and active subunits derived from the parent SAP may find a use in the receptor clustering experiments we are performing to get active genetic materials into a cell. We previously found that a synthetic peptide from SAP can support the attachment of many cell types to polystyrene surfaces by binding to cell surface sulfated polysaccharides. We now have identified two peptides from native SAP that bind heparin. The peptides are formed by digesting native SAP with cathepsin D. BSP, in addition to being involved in cell adhesion through an integrin, is a highly phosphorylated protein that probably plays a role in mineralization of bone. That certain integrins have been implicated in relocation of metastasizing cancer cells implied that the cyclic RGD motif in BSP can block the adhesion of breast cancer cells to bone cells in culture. We have found this to be true. CD4, in addition to binding strongly and specifically to gp120 of HIV-1 interacts closely with the beta chain of MHC Class II and in this role, CD4 allows the cells to function in a helper role during certain events in which the immune system is activated. It is not known if the gp120 binding part of CD4 is actually used in the everyday activities of CD4 or if, through some chance, CD4 simply has a region on its surface that is specifically recognized by gp120. We now can inhibit the binding of gp120 to CD4 with conformationally constrained peptides and peptomers that have been constructed.</p>		

Cooperating Units continued

Andreas Frei, Institut für Infectiologie, Westfälische Wilhelms-Universität, Münster, Germany

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00434-09 LCDO

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on HIV-1 Targeted Drug Delivery System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robey, F.A. Chief, PIU LCDO, NIDR

Others: Ivanov, Boris Visiting Fellow LCDO, NIDR
Liu, Mingfang Visiting Fellow LCDO, NIDR
Shi, Chong-Shan Visiting Fellow LCDO, NIDR

COOPERATING UNITS (if any)

Majorie Robert-Guroff, NCI; Peter Roller, NCI; Kay Jordan, OD, NIH; Marian Neutra, Harvard University; Andreas Frei, Institut für Infectiologie, Westfälische Wilhelms-Universität, Münster, Germany

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Peptide and Immunochemistry Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.59

PROFESSIONAL:

2.59

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The goal of this project has been to design and synthesize molecules derived from gp120 of HIV that bind to the CD4 receptor in the same fashion as the intact gp120. By binding to CD4, the peptide-based molecules competitively block the binding of gp120 to CD4 and, therefore, infection of cells by HIV-1. The materials we have made will be developed further as antagonists of HIV binding for in vivo uses, for targeting drugs and other therapeutics to CD4-bearing cells and as components of HIV vaccines. In addition, the peptide-derived materials are used to study cellular events that result from the binding of a CD4-specific peptide to the cell. We have observed peptide-induced signal transduction in CD4-bearing cells and the resulting cellular events provide us with insight into the pathogenesis caused by the chronic exposure of host cells to gp120. The understanding of the processes may lead to an understanding of the molecular basis behind AIDS in which the numbers of CD4-bearing cells decrease over several years in HIV-infected individuals. A necessary property of the active peptides is that they contain helical conformations for the activity of binding CD4 and for blocking the interaction of gp120 to CD4. The peptides are 15 to 18 amino acids in length and directly compete with the virus to block infection of cells in vitro. The D-forms of the peptides work as well as the L-forms indicating that there is probably great versatility in the recognition of HIV for binding CD4. We have learned that the hydrophobic surfaces of the various molecules are probably responsible for binding to CD4; this region of the helix is highly conserved among all the different strains of HIV-1, HIV-2 and SIV. Thus, the challenge has been to make materials that have an exposed hydrophobic surface but do not aggregate. Peptide aggregation is a problem in the field of hydrophobic drug development and we have this problem here. In addition, albumin is well-known to bind hydrophobic materials and our additional challenge has been to make a material that will not bind albumin and will not aggregate but will be recognized by CD4. Certain nonionic detergents appear to stabilize the helical conformation of the active peptide(s) and this may lead to active antagonists that could be components of spermicides. Spermicides are composed of nontoxic, nonionic detergents, so this finding argues compellingly for the introduction of clinical trials which test our helical peptides as HIV blocking agents. The ability of an adjuvant to preserve the conformation of an active peptide has been studied. The results clearly indicate that the adjuvants containing oil-like molecules such as the paraffin oil found in Freund's adjuvant will destroy the helical conformation by interfering with hydrophobic forces holding the helices together. In doing so, the adjuvants themselves disrupt conformation-dependent antibody biosynthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00479-07 LCDO

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms Responsible for Oncogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robbins, Keith C. Chief, MCBS LCDO, NIDR

Others: Matoskova, Brona Visiting Fellow LCDO, NIDR
Biesova, Zuzana Visiting Fellow LCDO, NIDR
Wong, William T. Staff Fellow LCDO, NIDR

COOPERATING UNITS (if any)

Paolo DiFiore, LCMB, DCE, NCI

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.31

PROFESSIONAL:

1.31

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this research program is to identify the mechanisms of neoplastic transformation in head and neck squamous cell carcinoma. We have focused on the epidermal growth factor receptor (EGFR) system because EGFR has been found to be constitutively active in high fraction of head and neck squamous cell carcinomas. It has been found that in addition to autophosphorylation, the activated receptor kinase also phosphorylates other specific intracellular proteins in the cell. It is believed that these intracellular substrates of EGFR kinase will then carry out the signal of EGF through a network of interacting proteins (or signal transduction pathways) that ultimately leads to cell proliferation or differentiation. Therefore, studies on these EGFR substrates will provide important information on the mechanisms of neoplastic transformation in head and neck squamous cell carcinoma. In collaboration with Dr. Pier Paolo Di Fiore of the National Cancer Institute, we have been investigating the nature of one of these receptor substrates, eps8. Our works have focused on the role of eps8 in EGFR signaling pathway and the biologic effect it imparts when switched on. We have evidence suggesting that eps8 is likely to play a role in the proliferation pathway of EGFR. We have also identified constitutively phosphorylation of eps8 in human tumor cell lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00480-07 LCDO

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Normal Physiologic Roles for Nonreceptor Protein-Tyrosine Kinases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robbins, Keith C. Chief, MCBS LCDO, NIDR

Others: Rivero, Octavio Visiting Fellow LCDO, NIDR
Teramoto, Hidemi Visiting Fellow LCDO, NIDR
Marcilla, Antonio Visiting Fellow LCDO, NIDR
Geiser, Jeanne Chemist LCDO, NIDR
Stephens, Edward Bio Lab Tech LCDO, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.11

PROFESSIONAL:

2.44

OTHER:

.67

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Our progress in the last year is in the line of research that we have been developing in the field of protein-tyrosine kinases. We have recently identified the major tyrosine phosphorylated protein observed after engagement of Fcγ receptors as p120^{c-b1}. We had already characterized this protein as an SH3 binding protein, because it specifically associates to the SH3 domains of p47^{nc}. In the myelomonocytic system we have observed that p120^{c-b1} associates with the SH3 domain of the non-receptor protein-tyrosine kinase p56^{lyn}. In another line of work, we have characterized cbl-b, a new proto-oncogene belonging to the c-b1 family, as an SH3 binding protein. Regulation of the phosphorylation of this protein by SH domain interactions is in progress. Protein-protein interactions have been analyzed in the tyrosine kinases p59^{hyn} and p55^{lgr} by mutational analysis. We have observed that these closely related kinases are very differently affected by mutations in very conserved residues of their SH2 and SH3 domains, suggesting that these domains regulate through various mechanisms the activity of these enzymes. During this last year we have also identified and characterized a second protein found to specifically associate to p47^{nc}. This gene has been recently cloned by Derry et al. (Cell 78:635-644) and denominated WASP, because it has been found to be mutated in WAS (Wiskott-Aldrich Syndrome) patients. We have characterized the protein expressed by this gene as an SH3 binding protein of 66 kDa, which is present in the nucleus, cytosol and membrane of myelomonocytic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00551-04 LCDO

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of G Proteins in Growth Control and Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Gutkind, J Silvio	Chief, MSU	LCDO, NIDR
Others:	Xu, Ningzhi	Visiting Associate	LCDO, NIDR
	Coso, Omar	Visiting Fellow	LCDO, NIDR
	Crespo, Piero	Visiting Fellow	LCDO, NIDR
	Teramoto, Hidemi	Visiting Fellow	LCDO, NIDR
	Vitale, Lynn	Pre Irta	LCDO, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular Signalling Unit

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

4.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our interest is in the molecular basis of cancer, and we have approached this problem by studying intracellular pathways which participate in the transduction of proliferative signals.

We have previously shown that mutated genes encoding certain classes of G proteins are as transforming as the most potent known oncogenes, and that cell surface receptors functionally coupled to these G proteins can induce malignant transformation in an agonist dependent manner. Growth promoting pathways activated by G protein-coupled receptors were shown to involve activation of the *ras* proto-oncogene and 72nd. The latter initiates activity from a serine-threonine kinase cascade that converges in the activation of extracellular signal-regulated kinases (ERKs) or MAP kinases, and ultimately regulates the expression of genes essential for proliferation. We have demonstrated that $\beta\gamma$ subunits of G proteins, not $G\alpha$, act in a *ras*-dependent manner to stimulate MAP kinases, converging at this level with the signaling route utilized by tyrosine kinase-growth factor receptors. Thus, activation of either type of receptor would be expected to elicit a similar response at the level of nuclear transcription factors. However, we have observed that activation of G protein-coupled receptors in NIH 3T3 cells induces a distinct pattern of expression of immediate early genes of the *jun* and *fos* family. These responses did not correlate with the activation of MAP kinases. We found that triggering G protein coupled receptors potently stimulate the activity of a novel family of enzymes closely related to MAP kinases, known as *jun* kinases (JNKs). In contrast, PDGF failed to activate JNK in these cells, although it stimulated MAP kinase to an even greater extent. We concluded that G protein-coupled receptors can signal through pathways leading to the activation of JNK, thus diverging at this level with those pathways utilized by receptors of the tyrosine kinase class. These observations led us to hypothesize that JNK and MAP kinase might be differentially regulated, and prompted us to investigate the biochemical route controlling JNK. Using the expression of an epitope-tagged JNK1 (HA-JNK) in COS-7 cells as a model system to explore the mechanism of activation of JNK, we found that Ras could weakly activate JNK, utilizing a pathway distinct from that regulating MAPK. In contrast, we showed that expression of mutationally activated forms of the small GTP-binding proteins Rac1 and Cdc42 initiate an independent kinase cascade leading to JNK activation, and have also shown that Rac and Cdc42 are an integral part of the signaling route linking G protein coupled receptors as well as receptors for inflammatory cytokines and epidermal growth factor to JNK. Furthermore, we have shown that JNK is potently activated by a number of naturally occurring human oncogenes, and recently published reports support a role for Rac1 in metastasis. Thus, we believe that our findings unveiling the existence of a novel signaling pathway communicating the cell surface to the nucleus have helped to identify a number of potential candidates as targets for therapeutic intervention in cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01DE00558-04 LCDO

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Oral Carcinogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Robbins, Keith C.	Chief, MCBS	LCDO, NIDR
OTHERS:	Cardinali, Massimo	Visiting Associate	LCDO, NIDR
	Yeudall, W. Andrew	Visiting Associate	LCDO, NIDR
	Jakus, Judit	Visiting Associate	LCDO, NIDR
	Geiser, Jeanne	Chemist	LCDO, NIDR
	Stephens, Edward	Bio Lab Tech	LCDO, NIDR
	Winn, Debbie	Chief, ASHAB	EODPP, NIDR
	Schwartz, Joel	Senior Dental Scientist	EODPP, NIDR

COOPERATING UNITS (if any)

John Ensley, Wayne State University; E. Kenneth Parkinson, Beatson Institute, Glasgow, UK;
Stephen S. Prime, Bristol University, UK.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Molecular and Cellular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.22

PROFESSIONAL:

3.55

OTHER:

.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The LCDO's Oral Cancer Program investigates etiology, diagnosis, treatment and prevention of oral tumors, placing a special emphasis on the most prevalent oral cancer, squamous cell carcinoma (SCC). One component of this effort involves identification of molecules that uniquely describe stages of progression from normal to malignant. The presence or absence of such molecules can be useful information (i) for diagnosis, (ii) in determining appropriate treatment modalities and (iii) in assessing the efficacy of chemoprevention protocols. Among the growth promoting molecules identified to date as markers of malignancy are the receptor for epidermal growth factor in combination with one or more of its agonists. Of the growth regulatory class of molecules, p53 is mutated and nonfunctional in a high percentage of SCCs, and an inhibitor of G1 cyclin kinases, p16, is compromised in all SCC cell lines studied to date. The expression of a second cyclin kinase inhibitor, p15, is less frequently altered. Furthermore, the ability of tumor cells to upregulate expression of a third cell cycle regulator, p21, in response to DNA damage or growth factor receptor signaling, is altered. Thus, the laboratory's repertoire of molecular markers heralding the malignant state continues to expand, and the functional consequences of dysregulation of these molecules during tumor development is becoming apparent. Assays developed for such molecules will be useful to others clinically and to us as intermediate biomarkers for assessing progress in chemoprevention settings, as well as providing an insight into the molecular mechanisms of oral squamous cell carcinogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00605-02 LCDO

PERIOD COVERED

October 1, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inhibitors of Hypusine Biosynthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Folk, John E. Chief, ECS LCDO, NIDR

Others: Park, Myung-Hee Research Chemist LCDO, NIDR
Wolff, Edith C. Expert LCDO, NIDR
Lee, Young-Bok Visiting Fellow LCDO, NIDR
Joe, Young Ae Visiting Fellow LCDO, NIDR

COOPERATING UNITS (if any)

Dr. H. Hanuske-Abel and others, Cornell University, Medical College- The New York Hospital, New York, NY.
Dr. B. Ganom and others, Cornell University, Medical Dept., Baber Lab. Ithaca, NY.

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.16

PROFESSIONAL:

2.16

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The unique amino acid hypusine occurs only in one cellular protein, eukaryotic protein translation initiation factor 5A (eIF-5A), and only at a single site. Because hypusine is formed posttranslationally and since both eIF-5A and its hypusine modification are essential for cell growth, we have aimed our attention toward inhibition of hypusine formation as a means of controlling eukaryotic cell proliferation. The two enzymatic steps in hypusine production, deoxyhypusine synthesis and deoxyhypusine hydroxylation, offer prime targets for intervention and inhibitors for the enzymes that catalyze these steps are being designed, synthesized and tested for their cellular effects.

Guanyl diamines modeled after quazatine, a quanylated polyamine with broad spectrum activity against seed-borne fungi and citrus mold which displayed some inhibition against the enzyme deoxyhypusine synthase, proved to be potent inhibitors of both this enzyme and cell proliferation and provided the basis for a recent U.S. patent entitled "Compositions and methods for inhibiting deoxyhypusine synthase and the growth of cells". That these inhibitors prevent growth of cells through intercellular inhibition of hypusine biosynthesis places them in a novel class of antiproliferative agents. Knowledge of the structural requirements for inhibitors of this enzyme has allowed design of agents for labeling of specific sites in its active center and has provided a degree of understanding of its mechanism of catalysis.

Certain metal chelating agents prevent hypusine formation through inhibition of deoxyhypusine hydroxylase. Suppression of hypusine formation by inhibition of this enzyme correlates with arrest of cell proliferation resulting in accumulation of cells in the late G1 phase of the cell cycle. Since certain of these agents also cause inhibition of prolyl 4-hydroxylase, arrest of proline-to-hydroxyproline conversion and consequently suppression of collagen secretion, they were tested for combined antiproliferative/fibrosuppressive effects on smooth muscle cells of human atherosclerotic coronary arteries. The positive combined effects have important implications for control of fibrosis in general and, in particular, for understanding the pathophysiology of restenosis, the recurrent closing of vessels of the heart following surgical reconstruction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00608-02 LCDO

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Deoxyhypusine Synthase: Purification, Characterization and cDNA Cloning

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Park, Myung Hee	Research Chemist	LCDO, NIDR
Others:	Wolff, Edith C.	Expert	LCDO, NIDR
	Lee, Young-Bok	Visiting Fellow	LCDO, NIDR
	Chung, Soo Il	Research Chemist	LCDO, NIDR
	Kang, Kee Ryeon	Visiting Fellow	LCDO, NIDR
	Folk, John E.	Chief, ECS	LCDO, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cellular Development and Oncology

SECTION

Enzyme Chemistry Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.08

PROFESSIONAL:

3.08

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An unusual amino acid, hypusine, which occurs in only one cellular protein, eukaryotic translation initiation factor 5A (eIF-5A), is intimately involved in cell proliferation. Hypusine biosynthesis occurs by way of two sequential post-translational modification reactions: i) deoxyhypusine synthesis by deoxyhypusine synthase and ii) deoxyhypusine hydroxylation by deoxyhypusine hydroxylase.

We have purified the first enzyme, deoxyhypusine synthase, to homogeneity after ~100,000-fold enrichment from rat testis. The purified enzyme displays a remarkably narrow specificity toward its substrates, spermidine, NAD, and the eIF-5A precursor protein and catalyzes deoxyhypusine synthesis in the complete reaction mixture. In the absence of the substrate protein, however, it carries out a partial reaction, the NAD-dependent cleavage of spermidine. The enzyme exists as a tetramer of 42 kDa subunits, with a pI of 4.75.

Using partial amino acid sequences from the rat testis enzyme, we were able to identify *YHRO68W* of *Saccharomyces cerevisiae* chromosome VIII as a gene for deoxyhypusine synthase. We have also cloned human cDNAs encoding a full-length deoxyhypusine synthase by immunoscreening of a HeLa cell cDNA library. After overexpression of the human deoxyhypusine synthase cDNA or the yeast cDNA of *YHRO68W* in *E. coli*, we purified the recombinant enzymes and characterized their physical and enzymatic properties. Deletion, insertion and site-directed mutagenesis studies are underway to gain insights into the active site structure and the structure-function relationship of this important enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00230-19 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Proteins in Tissue Architecture and Cell Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kleinman, Hynda K.	Section Chief	LDB, NIDR
Others:	Kibbey, Maura C.	Staff Fellow	LDB, NIDR
	Hoffman, Matthew	Visiting Fellow	LDB, NIDR
	Powell, Sharon	Staff Fellow	LDB, NIDR
	Malinda, Katherine	IRTA Fellow	LDB, NIDR
	Zivkovic, Sasa	Visiting Fellow	LDB, NIDR
	Cid, Maria	Guest Researcher	LDB, NIDR

COOPERATING UNITS (if any)

NIA, NIH, Jucker M; Univ. Conn, Klein, N; Univ. Colorado, Guzelian P; Children's Hospital, Petryshyn R; Georgetown U Med Sch, Wash DC, Dym M; FDA, Tosato G; Univ. Patras Med Sch, Patras Greece, Maragoudakis M.

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.65

PROFESSIONAL:

4.45

OTHER:

0.20

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The extracellular matrix has been found to be important in embryogenesis and in tissue repair. From in vitro studies using purified components, a better understanding of how cells adhere, migrate, proliferate, and differentiate in response to tissue and cell-specific matrix molecules has been established. We have found that the basement membrane, the extracellular matrix which underlies all epithelial cells and endothelial cells and surrounds nerve cells, promotes cell differentiation in vitro. When cultured on basement membrane, endothelial cells form capillary-like structures with a lumen, chondrocytes from cartilage, salivary cells form glands, etc. Our goal is to define the molecular and cellular events involved in this process. Our approach has been (1) to identify the biologically active matrix components, (2) localize active sites on the matrix component with synthetic peptides, (3) identify and characterize cellular receptors, (4) gain an understanding of the intracellular events involved in the biological response, and (5) identify genes induced by the extracellular matrix. Estrogens have been found to promote leukocyte adhesion to endothelial cell monolayers via an increase in endothelial cell selectin adhesion receptors. In addition, estrogens promote endothelial cell adhesion, growth, migration and tube formation in vitro and angiogenesis in vivo. Using the endothelial cell tube assay, a new role for proteases, interferon inducible protein 10 and collagen has been defined and may have important clinical uses in vessel repair. Subtractive cDNA cloning of endothelial cells on plastic vs basement membrane has identified several novel genes as well as thymosin B4 and calmodulin as induced during differentiation into vessels. The laminin-derived peptide SIKVAV promotes neurite outgrowth and binds a nucleolar protein which may shuttle to the nucleolus. Another active site LAVQLSIR on laminin has been identified for neurite outgrowth. These studies demonstrate cell type-specific responses to laminin domains.

Professional Personnel, continued

Ponce, Maria	IRTA Fellow	LDB, NIDR
Richard, Barbara	IRTA Fellow	LDB, NIDR
Thomas, Linda	Biologist	LDB, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00482-07 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tumor Growth and Metastases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kleinman, Hynda K.	Section Chief	LDB, NIDR
Others:	Kibbey, Maura C.	Staff Fellow	LDB, NIDR
	Kim, Woo H.	Visiting Fellow	LDB, NIDR
	Roque, Eva	Biologist	LDB, NIDR
	Song, Sang-Yong	Visiting Fellow	LDB, NIDR

COOPERATING UNITS (if any)

NIDDK, NIH, Lesoon-Wood L; NIA, NIH, Passaniti A; Lombardi Cancer Center, Wash DC, Thompson E; Catholic Univ, Wash DC, Tozeren A; Harvard Medical School, Meccurio A; Henry Ford Hospital, Bresalier R; Univ of Pittsburgh, Mokotoff M.

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.63

PROFESSIONAL:

1.88

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies are conducted to define the mechanisms involved in tumor growth and metastasis and to develop new animal models of human cancers. We have found that a basement membrane extract (Matrigel) when premixed with human tumor cells (which do not grow well in mice) promotes their incidence and growth. We have been able to culture new highly differentiated human tumor cell lines from the tumors grown in mice including certain colon cell lines. Laminin, a major basement membrane component, has been found to promote the malignant phenotype. Selection for adhesion to laminin was carried out with a human colon cancer cell line developed from a patient biopsy passaged in mice with Matrigel and the adherent cells were found to be highly malignant when injected with Matrigel. The laminin non-adherent cells formed few tumors which were highly differentiated.

Various biologically active laminin-derived synthetic peptides have been identified. Previously, we found that YIGSR (tyr-ile-gly-ser-ag) reduced tumor growth, metastases and angiogenesis. We now find multimeric forms of this peptide are more active than the monomers and are able to induce apoptosis. Another laminin-derived peptide containing SIKVAV from the A chain has been found to increase tumor growth, lung and liver colonization, and angiogenesis as well as collagenase IV activity and plasminogen activation. This peptide was found to promote angiogenesis in an in vivo model by increasing the recruitment of neutrophils. This peptide also increases monocytic macrophage PGE₂ and matrix metalloproteinase. Further screening of the G domain of laminin identified a peptide LQVQLSIR which increases melanoma metastases to the liver. Using this information and the newly developed models of human tumors, the development of new therapeutic strategies for cancer should be facilitated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00483-07 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Regulation and Function of Cartilage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Yoshihiko	Section Chief	LDB, NIDR
Others:	Yamada, Kenneth	Laboratory Chief	LDB, NIDR
	Krebsbach, Paul	Staff Fellow	LDB, NIDR
	Nakata, Ken	Visiting Fellow	LDB, NIDR
	Lee, Suk-Keun	Visiting Fellow	LDB, NIDR
	Bernier, Suzanne	Visiting Fellow	LDB, NIDR
	Watanabe, Hideto	Visiting Fellow	LDB, NIDR
	Rhodes, Craig	Biologist	LDB, NIDR

COOPERATING UNITS (if any)

Shriner's Hospital, OR; Aichi Medical University, Nagoya, Japan; NIDDK, NIH

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.7

PROFESSIONAL:

3.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Cartilage is a highly specialized connective tissue consisting of a sparse number of chondrocytic cells embedded in an extensive extracellular matrix. The biomechanical characteristics of the cartilage extracellular matrix are particularly suited to bearing a compressive load. The purpose of this project is to understand the structure and function of cartilage components and to identify the mechanisms underlying cartilage formation. We have also initiated a genome project to identify genes crucial for craniofacial and tooth development. Collagen II is a major fibrillar collagen in cartilage. We previously identified an enhancer in the first intron of the collagen II gene which increased transcription of the gene. We have identified a promoter element located close to the transcription initiation site which requires the enhancer-mediated transcription of the type II collagen gene. We have identified a 100 bp sequence as the minimum size of the enhancer which contained several sequence motifs homologous to the regulatory region of the link protein gene. We have cloned several protein factors bound to the enhancer. One of them is the C-propeptide of type II collagen. Our results suggest that the C-propeptide down-regulates the transcription of the type II collagen gene through negative feedback mechanisms. We have identified an enhancer of the link protein gene. This region contains a sequence homologous to the enhancer sequence of the collagen II gene and suggests that a common factor is involved in coordinate transcription of both link protein and collagen II genes in chondrocytes. A genome project for craniofacial and tooth development has been initiated in collaboration with the Developmental Mechanisms Section. The goal of this project is to understand the molecular mechanisms underlying craniofacial and tooth development. We have identified a novel gene specific to ameloblasts from the incisor cDNA library. We named this new gene product "ameloblastin" because its expression is specific to ameloblasts. Expression of ameloblastin mRNA is most intense in secretory-stage ameloblasts, similar to amelogenin mRNA. Ameloblastin mRNA is also expressed in premature stage ameloblasts and in retrogressive ameloblasts where amelogenin mRNA is rarely expressed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00484-07 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models of Connective Tissue Disease in Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Yoshihiko	Section Chief	LDB, NIDR
Others:	Watanabe, Hideto	Visiting Fellow	LDB, NIDR
	Nakata, Ken	Visiting Fellow	LDB, NIDR
	Takami, Hiroya	Visiting Fellow	LDB, NIDR
	Krebsbach, Paul	Staff Fellow	LDB, NIDR
	Strong, David	Biological Lab Tech	LDB, NIDR

COOPERATING UNITS (if any)

Osaka University, Osaka, Japan; Wistar Institute, Philadelphia, PA; HSP Research Institute, Osaka, Japan; Mark Sharp & Dohme Research Lab., NJ

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.9

PROFESSIONAL:

1.9

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Creation of gene targeted mutations and the introduction of dominant mutations in transgenic mice are useful for understanding the function of proteins in development and for screening animal models of various diseases for potential therapies. The purpose of this project is to create animal models in mice for studying the molecular basis of genetic and acquire diseases associated with connective tissues. These models will also be useful in elucidating the role of these proteins in development. Genes for the basement membrane and cartilage components have been cloned and the exon-intron structure of these genes have been characterized. Mouse laminin $\alpha 2$, $\beta 3$, and $\gamma 2$ chains have been cloned and sequenced. Murine dystrophin muscularis-2J (dy2J) is an autosomal recessive disease characterized by muscle degeneration and developmental dysmyelination of peripheral nerves. We have identified the defect in the laminin $\alpha 2$ chain of dy2J mice. The $\alpha 2$ -chain cDNA sequence, amplified by RT-PCR from dy2J mice identified a novel, predominant transcript with a 171 base in-frame deletion. This was confirmed with a splice donor site mutation in the $\alpha 2$ chain gene of dy2J mice. Translation of this variant transcript would result in the expression of a truncated $\alpha 2$ chain with a 57 amino acid deletion and also a substitution of Gln by Glu at residue 91 in the N-terminal domain VI, which is presumed to be involved in the self aggregation of laminin and in cell attachment. The mutant $\alpha 2$ chain could disrupt the formation of the laminin network or the binding to Schwann cells and thereby lead to muscle degeneration. To examine cell-type specific activity of the type II collagen enhancer and promoter in vivo, we have prepared lacZ reporter gene constructs with combinations of various sizes of the type II promoter and enhancer containing intron fragments and have micro-injected them into mouse embryos to create transgenic mice. We have established homozygous mouse lines with most of these constructs and have been analyzing expression patterns of the lacZ gene in these mice. Mutations in the endogenous genes for basement membrane and cartilage proteins have been created by homologous recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00485-07 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Regulation and Function of Basement Membrane

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Yoshihiko	Section Chief	LDB, NIDR
Others:	Nomizu, Motoyoshi	Visiting Associate	LDB, NIDR
	Utani, Atsushi	Visiting Fellow	LDB, NIDR
	Bernier, Suzanne	Visiting Fellow	LDB, NIDR
	Takami, Hiroya	Visiting Fellow	LDB, NIDR
	Tanaka, Masahiko	Special Volunteer	LDB, NIDR
	Thomas, Linda	Biologist	LDB, NIDR

COOPERATING UNITS (if any)

Max-Planck-Institute, Munich, Germany; Univ. of Pittsburgh, Pittsburgh, PA; Univ. of Genova, Italy; INSERM U49, France; Univ. of Iowa, Iowa City, IA

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Molecular Biology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.35

PROFESSIONAL:

4.15

OTHER:

0.20

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Basement membranes are thin sheets of extracellular matrix surrounding most tissues and play a critical role in tissue development, repair, and maintenance. The aim of this project is to understand the molecular mechanisms underlying the role of the basement membranes in these biological processes. Basement membranes contain a unique set of proteins, such as collagen IV, laminin, perlecan and nidogen/entactin. Laminin is a family of heteromeric glycoproteins specific in basement membranes and has a number of biological activities. Mouse laminin $\beta 3$ and $\gamma 2$ chains were cloned and their primary structure determined. Their tissue-specific expression was also studied by in situ and Northern hybridization. The diversity of laminin chains has raised the question of how the chains are selected and assembled into intact laminin molecules. The assembly of laminin chains to double- and triple-stranded structures was studied with recombinant proteins and synthetic peptides. We have found that chain selection is controlled by the C-terminal region of the α -helical domain of each chain. We found that thermodynamically stable trimer formation requires a minimum sequence of about 50 amino acids, including the core sequence necessary for assembly. Circular dichroism (CD) analysis revealed that $\beta 1$ peptide homodimers are more stable than $\beta 1/\gamma 1$ heterodimers. However, a mixture of $\beta 1$ and $\gamma 1$ peptides preferentially forms $\beta 1/\gamma 1$ heterodimers. The relatively unstable structure of $\beta 1/\gamma 1$ heterodimers can facilitate the formation of stable heterotrimers with the α chain. We have screened a series of overlapping peptides that cover most of the domain G of laminin $\alpha 1$ chain (amino acids residues 2111-3060) to identified bioactive sites. Five new peptides (AG-10, AG-22, AG-32, AG-56 and AG-73) had cell attachment activity. AG-32 and AG-73 were chemotactic and promoted neurite outgrowth. We have cloned and characterized a new rhoGAP protein, p190B. Our results suggest that signals from extracellular matrix molecules may influence the activity of p190 and rho proteins. We have found that transcription of the collagen IV genes in F9 cells is regulated not only by enhancers and protein factors, but also by the chromatin structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00524-05 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functions and Developmental Regulation of Matrix Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Kenneth M	Chief	LDB, NIDR
Others:	Brown, Karen E	IRTA Fellow	LDB, NIDR
	Gutkind, J Silvio	Unit Chief	LCDO, NIDR
	Kim, Lawrence T	IRTA Fellow	LDB, NIDR
	Lafrenie, Robert M	Visiting Fellow	LDB, NIDR
	Miyamoto, Shingo	Visiting Fellow	LDB, NIDR
	Thomas, Linda	Biologist	LDB, NIDR

COOPERATING UNITS (if any)

University of Tennessee (Donaldson D); Institut Curie, France (Thierry JP); Kyoto Univ., Japan (Takeichi M); Univ. British Columbia (Larjava H), Univ. Hosp., Nijmegen, Netherlands (Danen E).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.75

PROFESSIONAL:

2.55

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Integrins and other cell surface receptors for extracellular matrix proteins such as fibronectin function in embryonic development and wound healing. Previous studies demonstrated key roles for integrins in early development. Our recent findings suggest novel potential roles in tooth development and wound repair, and they provide insights into integrin mechanisms of action. In tooth development, we found rapid changes in $\beta 5$ integrin mRNA expression and unusually prominent expression of the αv integrin. Differences between the specific expression patterns of $\beta 3$ and $\beta 5$ integrin subunits in embryos and the generally diffuse patterns of αv and $\beta 1$ subunits correlated with their different integrin partner specificities: the latter two pair with many other subunits, while the former are specific. A collaborative study of periodontal disease tissue revealed disruption of the organization of $\beta 1$ integrins and extracellular proteins. Another localization study on wound repair revealed differences between types of epithelial repair, one requiring activation of keratinocytes, but another not. Studies of integrin mechanisms of action focused on their binding to target ligands such as fibronectin and subsequent responses. Although binding of the human fibronectin receptor $\alpha 5 \beta 1$ generally requires a two-site mechanism including a synergy site, maximal activation of this integrin allows it to bypass this requirement, suggesting a novel regulatory mechanism. The roles of integrin ligand occupancy, aggregation, and a combination of these two events were tested using natural ligands, soluble peptides, immobilized multivalent peptides, and monoclonal antibodies. Distinct roles were identified for occupancy versus aggregation, and synergistic effects of the combination in controlling receptor location, signaling, and association with different classes of cytoskeletal molecules. These approaches provide novel tools for understanding how extracellular molecules regulate cellular functions. Because alterations in integrin function may contribute to a variety of human congenital defects and affect wound healing, these studies also provide an opportunity to identify new pathways as targets for potential therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00525-05 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms and Regulation of Cell Adhesion, Migration, and Morphogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Kenneth M	Chief	LDB, NIDR
Others:	Aota, Shin-ichi	Visiting Associate	LDB, NIDR
	Brown, Karen E	IRTA Fellow	LDB, NIDR
	Savagner, Pierre	Special Volunteer	LDB, NIDR
	Lee, Chong-Chou	Special Volunteer	LDB, NIDR
	Komoriya, Akira	Special Volunteer	LDB, NIDR
	Akiyama, Steven K	Research Chemist	LDB, NIDR
	Yaen, Christopher	Biological Aid	LDB, NIDR

COOPERATING UNITS (if any)

Dept. Anatomy, Univ. Pennsylvania (Lash J), Dept. Dermatology, SUNY Stony Brook, NY (Clark R), Dept. Clinical Microbiology, Hebrew Univ., Jerusalem, Israel (Hanski E), CNRS, Institut Curie, Paris (Thierry JP), NCHGR (Pavan W).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.7

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cell adhesion, migration, and morphogenesis are crucial events in craniofacial development, and errors in them can produce congenital anomalies. Similar processes appear to be important for normal adult wound repair. Molecules that mediate or regulate these processes are being characterized. Fibronectin is important for both morphogenesis and epithelial wound healing, e.g. for migration of craniofacial neural crest cells. Regions of fibronectin essential for cell adhesion and migration were characterized in detail by site-directed mutagenesis, homology scanning, and monoclonal antibody approaches. The sequence Pro-His-Ser-Arg-Asn was shown to be essential for cell adhesion mediated by the $\alpha 5 \beta 1$ fibronectin receptor, and the Arg residue was crucial. This information should facilitate the rational design of novel bioadhesives and inhibitors. Although fibronectin could not be implicated in embryonic nephric duct extension, NCAM polysialic acid appeared to be involved. Vitronectin is another major extracellular adhesion molecule. Analysis of mRNA expression was striking in the spinal cord floor plate of mouse embryos. This structure is important for neuronal development, and vitronectin may be a novel effector molecule. Adhesion, migration, and morphogenesis are regulated by a variety of factors including cytokines and receptors, such as the c-met proto-oncogene receptor. We discovered and further characterized a novel spliced version of this tyrosine kinase receptor, which we found to be a multi-functional regulatory region involving PKC responsiveness, PI 3-kinase binding, and tyrosine phosphorylation. This and other receptors have been implicated in the inter-conversion of epithelia and mesenchyme in development. We showed novel regulation of splicing and function of this family of receptors. We have identified two candidate genes involved in the process of epithelial-mesenchymal transition, one of which appears to trigger this important process. These studies should provide a molecular understanding of the regulation of these important but complex morphogenetic processes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00559-04 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Activities of HIV Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Kleinman, Hynda K.	Section Chief	LDB, NIDR
Others:	Richard, Barbara A.	IRTA Fellow	LDB, NIDR
	Roque, Eva	Biologist	LDB, NIDR

COOPERATING UNITS (if any)

St. Louis University, IL, Green M, Lowenstein M; NINDS, NIH Lieberman D, Oldfield E.

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Cell Biology Section and Developmental Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.65

PROFESSIONAL:

0.40

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Patients with HIV infection who have AIDS are subject to an unexplained dementia even though virus is not observed in the brain. It is proposed that a soluble factor released from the virus may be affecting patients. We find that the HIV viral protein Tat promotes neural cell adhesion in vitro and blocks laminin-mediated process outgrowth. These events are mediated by a 90 kDa Tat receptor and were localized to a 9 amino acid sequence in Tat. Direct injection of Tat into the brains of rats caused impaired motor function and destruction of large amounts of brain tissue. High doses resulted in death. These data demonstrate that Tat has a strong effect on neural cells and suggest a possible mechanism to explain the neurologic changes and dementia observed in patients with AIDS. Infection of T cells with HIV-1 stimulated invasiveness through basement membrane, and synthesis of the 92 kDa type IV collagenase (gelatinase B). Monocyte infection by HIV-1 resulted in substantial increases in cell-cell adhesion mediated primarily by the integrin $\alpha_1\beta_2$. Increased adhesion of HIV-infected monocytes to a variety of endothelial cell types was followed by marked disruption of endothelial cell layers and increased vascular permeability, accompanied by increased expression of the 92 kDa metalloproteinase gelatinase B. This endothelial disruption was inhibited strongly by protease inhibitors including the TIMPs. These findings suggest the hypothesis that HIV-1 infection may activate blood monocytes to adhere to endothelium in aggregates, then to secrete increased amounts of protease activity resulting in endothelial disruption, thereby permitting invasion of HIV-infected cells into tissues. In preliminary studies, the HIV product Tat by itself induced secretion of the 92 kDa gelatinase B in dose-dependent fashion. These novel findings may provide a mechanism for metalloproteinase induction in HIV-infected cells, and they suggest that creative new approaches using specific metalloproteinase inhibitors to block tissue invasion might ultimately provide new insights into AIDS pathogenesis and preventive therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00560-04 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Functional Analysis of Membrane-Cytoplasmic Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Yamada, Kenneth M	Chief	LDB, NIDR
Others:	De Nichilo, Mark O	Visiting Fellow	LDB, NIDR
	Katz, Ben-Zion	Visiting Fellow	LDB, NIDR
	Kim, Lawrence T	IRTA Fellow	LDB, NIDR
	Miyamoto, Shingo	Visiting Fellow	LDB, NIDR
	Akiyama, Steven K	Research Chemist	LDB, NIDR

COOPERATING UNITS (if any)

Fogarty International Center, NIH (Geiger B); Laboratory of Biochemistry, National Cancer Institute (Vinson C)

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.8

PROFESSIONAL:

2.8

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interface between the plasma membrane and the cytoplasm of cells is the site for bidirectional information transfer between extracellular and intracellular processes in functions as diverse as adhesion and tissue formation, cell and tissue movements, and the regulation of gene expression, cell growth, and the cytoskeleton. Coordination and spatial organization of cellular structures associated with this interface, such as adhesion sites, the intracellular actin-based cytoskeleton, and signal transduction systems, are thought to be crucial for key steps in embryonic development, wound healing, and differentiated tissue function. Integrins are the major class of receptors used by cells to interact with the extracellular matrix, and they mediate cell adhesion and transmembrane signaling from extracellular matrix molecules to the interior of cells. The roles of integrin cytoplasmic domains and of the molecules that interact with integrins are being explored using molecular biology and biochemical methods. Functions of isolated domains and of individual cytoplasmic molecules are being tested using chimeric receptors containing a reporter domain consisting of a subunit of the interleukin-2 receptor and various molecules. The $\beta 1$, $\beta 3$, and to some extent $\beta 5$ integrin cytoplasmic domains were found to mediate targeting of receptors to adhesion sites of cells. When overexpressed, the $\beta 1$ and $\beta 3$ domains could inhibit integrin-mediated adhesion, migration, signaling from cytoplasm to external domains, and extracellular matrix assembly in "dominant negative" fashion. A distinct hierarchy of cytoplasmic responses was identified in analyses of the requirements for controlling the localization of specific molecules. Distinct roles were found for aggregation of integrins with or without ligand occupancy, intracellular tyrosine phosphorylation, and actin-based cytoskeletal integrity. Signaling molecule aggregation and signal transduction via the ERK and JNK classes of MAP kinase pathway formed another subset of these responses. Chimeras containing individual cytoskeletal and signal transduction molecules are being tested as mediators of steps in this hierarchy of responses. Characterization of these distinct biological steps provides novel tools for understanding the transmembrane spatial control of adhesion/signaling complexes that are essential for coordinating the complex rearrangements and final organization of oral, facial, and other developing tissues. These interactions are also likely to be important for adult tissue repair.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00563-04 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Cell-Substrate Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Akiyama, Steven	Research Chemist	LDB, NIDR
Others:	Yamada, Kenneth	Laboratory Chief	LDB, NIDR
	Aota, Shin-ichi	Visiting Associate	LDB, NIDR
	Kioka, Noriyuki	Visiting Fellow	LDB, NIDR
	Torchia, Dennis	Section Chief	MSB, NIDR
	Copie, Valerie	IRTA Fellow	MSB, NIDR
	Do, Kimlien	Biological Aid	LDB, NIDR

COOPERATING UNITS (if any)

Georgetown University Medical Center (Chen WT), University of Manchester (Humphries M), MSB, Lombardi Cancer Center (Dickson RB), New York Medical College (Godfrey HP), NIEHS, NIH (Olden K).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

LDB, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.7

PROFESSIONAL:

2.3

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fibronectin and integrin receptors play important roles in such processes as embryonic development, wound healing, and the progression of cancer. Techniques involving monoclonal antibodies, molecular and cell biology, and physical biochemistry are used to elucidate molecular mechanisms of fibronectin-receptor interactions with the long-term goals of producing novel bioadhesive substrates and developing rational bases for medical intervention in diseases involving abnormal cellular adhesion and migration. The central cell-binding region of fibronectin requires two distinct sequences for activity: an RGD sequence and a synergistic sequence. A 20 kDa fibronectin cell adhesive fragment of human fibronectin and containing both the RGD and synergy cell adhesive sites has been cloned and expressed. Although the fragment is highly active in soluble form, it has only poor activity when adsorbed directly onto plastic substrates. Full cell adhesive activity can be recovered if the 20 kDa fragment is bound to a non-inhibitory anti-fibronectin antibody pre-adsorbed onto plastic, suggesting that proper presentation of small fibronectin fragments may be important for maximal cell adhesive activity. The structure of a similar murine 20 kDa cell adhesive fragment in solution is being determined by NMR spectroscopic techniques. This is one of the largest polypeptide high-resolution structures attempted to date. Information obtained so far indicates that the synergy and RGD sites do not interact. Certain monoclonal antibodies that bind to integrins can up-regulate their ligand-binding activity. One such activating antibody designated 12G10, appears to bind to a "ligand induced" conformation. The role of the human $\alpha 5 \beta 1$ integrin in experimental metastasis has been analyzed using inhibitory anti- $\alpha 5$ and anti- $\beta 1$ monoclonal antibodies. Both antibodies inhibit metastasis of human breast carcinoma cells in athymic nude mice. The $\alpha 5 \beta 1$ integrin may be functioning in several steps of the metastatic cascade including in tumor cell attachment, migration, and extravasation. The modulation of the function of the $\alpha 2 \beta 1$ integrin by a panel of human breast carcinoma cells has been examined. This integrin was found to be present and to function in cell adhesion to collagen on all of the cells tested. The non-malignant and/or well differentiated cells also used the $\alpha 2 \beta 1$ integrin for adhesion to laminin. In contrast, highly invasive and/or poorly differentiated cells could not, suggesting that the ligand specificity of the $\alpha 2 \beta 1$ integrin could be regulated during malignant progression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00650-01 LDB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV Regulation of Adhesion Molecules and Proteases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Yamada, Kenneth M Chief LDB, NIDR
Others: Lafrenie, Robert M Visiting Fellow LDB, NIDR

COOPERATING UNITS (if any)

Division of Transfusion Transmitted Diseases, CBER, FDA (Dhawan S, Hewlett I).

LAB/BRANCH

Laboratory of Developmental Biology

SECTION

Developmental Mechanisms and Disorders Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

HIV infection alters a variety of cellular functions, including cell adhesion and protease secretion. The goals of this project are to determine the functions of integrins and proteases in the response of human monocytes to HIV infection, the mechanisms by which HIV regulates these molecules, their roles in AIDS pathogenesis, and approaches to inhibiting these alterations. Infection of human monocytes by HIV-1 resulted in marked alterations in cell-cell adhesion that could be attributed to changes in levels and function of β_2 integrins. Specifically, infected monocytes aggregated much more with other monocytes (homotypic adhesion) and displayed enhanced adhesion to microvascular endothelial monolayers (heterotypic adhesion). Infected monocytes also displayed increased mRNA levels and secretion of the matrix metalloproteinase MMP-9 (gelatinase B). Endothelial cell monolayers with attached monocytes became disrupted morphologically, and they showed increased permeability to passage of a radiolabeled albumin marker; these effects were inhibited by the metalloproteinase inhibitors TIMP-1 and TIMP-2. We tested the ability of the HIV regulatory molecule Tat alone to affect integrin function and protease induction. Tat treatment mimicked many of the effects of HIV-1 infection. It induced homotypic cell-cell aggregation of treated (but uninfected) monocytes, and it stimulated monocyte adherence to both untreated and TNF α -treated microvascular endothelial cell monolayers. The effects could be attributed to changes in the synthesis, surface expression, and function of β_2 integrins. These effects on adhesion could be inhibited by antibodies against the β_2 integrin subunit and its ICAM-1 counter-receptor. Moreover, HIV-Tat treatment alone induced a dramatic induction of MMP-9 synthesis. These studies suggest roles for integrins and proteases in the pathogenesis of AIDS. The changes are consistent with monocyte activation, with enhanced ability to adhere to and disrupt endothelium by means of enhanced integrin and protease expression. We propose that such HIV-activated cells would have an increased propensity to migrate into tissues; such tissue macrophages are known to be major reservoirs of HIV virus. These findings suggest that approaches using specific inhibitors of soluble Tat function or of monocyte activation and invasion might provide new insights into AIDS pathogenesis and preventive therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00034-27 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Histamine Release

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Siraganian, Reuben P.	Chief, RASTS	LI	NIDR
Berenstein, Elsa	Microbiologist	LI	NIDR
Bhattacharyya, Siba	Visiting Associate	LI	NIDR
Hook, William A.	Guest Researcher	LI	NIDR
Kimura, Teruaki	Visiting Fellow	LI	NIDR
Okazaki, Hidetoshi	Visiting Fellow	LI	NIDR
Sagawa, Kenji	Guest Researcher	LI	NIDR
Zhang, Juan	IRTA Fellow	LI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.15

PROFESSIONAL:

3.65

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Histamine release from mast cells and basophils is being studied as an immunological mechanism involved in inflammation. The activation of these cells by immune cell surface receptors is also a model for signal transduction and cell secretion. Besides cell surface receptors, the cells can be activated by other secretagogues such as the calcium ionophore A23187. The emphasis the last few years has been in understanding the role of protein tyrosine phosphorylations in the signalling. The cultured rat basophilic leukemia cells are used as one of the main models for these studies.

Professional Personnel, continued

Bader, Greta	Biologist	LI	NIDR
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00046-24 LI

PERIOD COVERED

October 01, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Normal and Aberrant Mechanisms of Inflammation, Repair and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: McCartney-Francis, Nancy	Special Expert	LI, NIDR
Mizel, Diane	Chemist	LI, NIDR
Frazier-Jessen, Michelle	IRTA Fellow	LI, NIDR
Panek, Robert	IRTA Fellow	LI, NIDR
McGrady, George	Biologist	LI, NIDR
Ambrose, Olevia	Biological Technician	LI, NIDR
Wahl, Sharon M.	Chief, CIS	LI, NIDR

COOPERATING UNITS (if any)

Ashok Kulkarni, NINDS J.M. Ward, NCI; J. McCarthy, University of Minnesota; R. Redman, VA Hospital, Washington, D.C.; Pam Manning, Monsanto

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

5.35

PROFESSIONAL:

3.0

OTHER:

2.35

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this research program is to define the molecular mechanisms that regulate the inflammatory process. One effector molecule that has been implicated as a regulator of inflammatory responses is transforming growth factor beta (TGF- β). The TGF- β 1(-/-) mouse provides a model to explore the in vivo regulatory role of TGF- β 1. These mice develop multifocal inflammatory lesions in vital organs and die within 3-4 weeks. TGF- β 1 (-/-) mice initially exhibit increased poly- and autoreactive antibodies of the IgM isotype. As animals become increasingly symptomatic, this initial wave of IgM antibodies is replaced by those of an IgG isotype, a pattern consistent with autoantigen-driven immune responses. Plasma IL-6 and IFN- γ levels and mRNA expression (by semi-quantitative RT-PCR) for IFN- γ , IL-4, IL-6 and IL-10 are elevated in TGF- β 1 (-/-) lymphoid and target organs, suggesting potential mechanisms whereby lack of TGF- β 1 could lead to B cell activation and polyclonal expansion in TGF- β 1 (-/-) mice. The production of antibodies leads to autoimmune-like lesions in several tissues. Inflammatory sites within the salivary gland, characterized by periductal lymphocytic infiltration and increased proliferation, cytokine mRNA expression, and IgG-positive cells resemble lesions of Sjögren's syndrome. Glandular atrophy and loss of acini with reduced saliva production appear to contribute to the wasting syndrome characteristic of the TGF- β 1(-/-) mice. In an attempt to block inflammation and rescue the mice, TGF- β 1(-/-) mice were treated with synthetic fibronectin (FN) peptides. Daily systemic injection of FN peptides not only prevented tissue infiltration and weight loss in TGF- β 1(-/-) mice, but also reversed the acinar and ductal derangements, suggesting that the inflammation compromises glandular structure and function. These TGF- β 1(-/-) mice provide an important model of autoimmune disease which can be utilized in the design of therapeutic interventions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00199-19 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vitro Studies of Secretory Cell Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Oliver, Constance	Guest Research Biologist	LI	NIDR
Siraganian, Reuben	Chief, RAST	LI	NIDR
Swaim, William D.	IRTA Fellow	LI	NIDR
Weedon, Lynda	Biologist	LI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.80

PROFESSIONAL:

1.30

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The secretory process in several cell types is being investigated. The rat basophilic leukemia cell line (RBL-2H3) and other cultured cells are used to study various aspects of endocytic and secretory processes. Emphasis is on the use of morphological, cytochemical and biochemical characterizations in these cultured cells. Events involved in receptor activation and signal transduction are being investigated, as well as endocytic and secretory pathways. During the last year emphasis has been on the study of the biochemical changes induced by the binding of a monoclonal antibody to a cell surface ganglioside.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00290-16 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Production of Hybridomas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Siraganian, Reuben P.	Chief, RASTS	LI	NIDR
Fischler, Cynthia	Biologist	LI	NIDR
Weedon, Lynda	Biologist	LI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.68

PROFESSIONAL:

.10

OTHER:

.58

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hybridomas are being produced which secrete monoclonal antibodies to defined antigen specificity. In the past hybridomas have been selected that recognize the high affinity IgE receptor, its components and associated cell surface proteins that are involved in signal transduction. Monoclonal antibodies to several other cell surface proteins have also been selected. During the last year monoclonal antibodies were produced that recognize phosphorylated proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00441-09 LI

PERIOD COVERED

October 01, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Chronic Immune/Inflammatory Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Zagorski, John	Senior Staff Fellow	LI, NIDR
	Wahl, Sharon M.	Chief, CIS	LI, NIDR
	McCartney-Francis, Nancy	Special Expert	LI, NIDR

COOPERATING UNITS (if any)

J.B. McCarthy, Univ. of Minnesota; W. Leadbetter, Orthopedic Center; U. Skaleric, Slovenia, Pam Manning, Monsanto

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Administration of group A streptococcal cell wall (SCW) peptidoglycan-polysaccharide complexes induces arthritis, liver fibrosis, and spleen cell anergy in genetically susceptible rodents and provides a model to explore all phases of an immune response and the consequences of its dysregulation. One objective of this research is to identify known and novel molecules expressed during the development of inflammation and we have focused on the chemokine superfamily using *in vitro*, *in vivo* and *ex vivo* approaches. Cultured synovial fibroblasts express specific chemokine genes, including CINC and MCP-1, but not others, under pro-arthropathic conditions, implicating selective recruitment of leukocyte populations. Moreover, by engineering mutated forms of CINC and other chemokines with sequence modifications predicted to produce receptor antagonism (RA), it may be possible to block chemotaxis and control leukocyte accumulation at sites of inflammation.

To identify additional genes expressed during SCW-induced inflammation, we have used differential display PCR to compare gene expression in granulomatous and normal livers. Several partial cDNAs representing differentially expressed and novel genes have been cloned and sequenced. Continued definition of these experimental pathways may provide insight into human chronic inflammatory disorders including arthritis, injury associated disorders, and periodontitis. For example, our previously demonstrated involvement of the NO pathway in this model is consistent with the elevated nitrite levels we detect in synovial fluids from patients with rheumatoid arthritis and gingival fluids in periodontal disease. By immunohistochemistry, the inflamed human tissue samples have increased numbers of T cells, B cells and macrophages, and positive staining for iNOS protein. Since these data provide evidence for the existence of inducible NO biosynthesis in human inflammatory disease, the use of inhibitors selective for iNOS may be beneficial.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00456-08 LI

PERIOD COVERED

October 01, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Signal Transduction in the Monocyte/Macrophage

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Wahl, Larry	Senior Investigator	LI, NIDR
	Zhang, Yahan	NRC	LI, NIDR
	Mertz, Prema	Staff Fellow	LI, NIDR
	Shankavaram, Uma	IRTA Fellow	LI, NIDR
	Friedman, Jason	Biologist (Gift Fund)	LI, NIDR
	Wahl, Sharon M.	Chief, CIS	LI, NIDR

COOPERATING UNITS (if any)

I. Katona, USUHS; W. Stetler-Stevenson, NCI; D. DeWitt, Mich. State Univ.; H. Sage, Univ. of WA; J. Suttles, E. Tenn State University

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.51

PROFESSIONAL:

2.26

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This past year the focus of the research has been on the manner in which factors found at an inflammatory site may impact on the PGE2-cAMP dependent signal transduction pathway involved in MMP production by monocytes. The extracellular matrix component SPARC/osteonectin was shown to induce PGHS-2, eicosanoid and MMP production which was primarily attributed to a peptide (3.2) found in domain III of SPARC. SLPI, a prominent component of saliva, was shown to be a potent inhibitor of PGHS-2 and MMP production by monocytes at concentrations of 0.1 to 10 µg/ml, well within the physiological range of 1 to 20 µg of SPARC/ml of saliva. In our studies on the role of cytokines in the regulation of PGHS-2 and MMP, TNFα was shown to enhance LPS-induced PGHS-2 and MMP production. When added to monocyte cultures alone, TNFα failed to induce PGHS-2 or interstitial collagenase but did increase the basal levels of gelatinase B, indicating differential regulation of this MMP. The role of tyrosine kinases in the regulation of the PGE2-cAMP dependent pathway leading to MMP production by monocytes is being evaluated. The tyrosine kinase inhibitor herbimycin A was shown to inhibit the induction of PGHS-2 and MMP. This inhibition occurred at an early step in the signal transduction process since PGE2 or Bt2cAMP restored PGHS-2 and MMP production in herbimycin A inhibited monocyte cultures. Phosphatases also play an important role in signal regulation. Addition of the phosphatase inhibitor okadaic acid resulted in a significant enhancement of LPS-induced PGHS-2 and MMP. The presence of these mediators in tissues from periodontal lesions are being evaluated by immunohistochemistry and in situ hybridization. Areas of connective tissue destruction contained large numbers of PGHS-2 positive macrophages as well as some positive fibroblasts and epithelial cells. Interstitial collagenase was localized to the macrophages, fibroblasts, and epithelial cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00513-06 LI

PERIOD COVERED

October 01, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Monocytes in AIDS and as Targets for Antiviral Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: McNeely, Tessie	Sr. Staff Fellow (CRADA)	LI, NIDR
Tucker, Christina	Pre-IRTA Fellow (CRADA)	LI, NIDR
Wahl, Sharon M.	Chief, CIS	LI, NIDR

COOPERATING UNITS (if any)

S. Eisenberg, Synergen, Boulder, CO; Jan Orenstein and Paula Worley, GWU; J.B. McCarthy, Univ. Minnesota; P.D. Smith, Univ. Alabama; Ed Janoff, Univ. Minnesota

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

1.1

OTHER:

1.00

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monocytes and macrophages express CD4 on their surface, are targets for HIV-1, and may serve as a reservoir for the virus. Ongoing studies focus on the role this population plays in the progression of HIV-1 disease and as targets for antiviral therapy. Human saliva contains potent anti-HIV-1 factor(s), consistent with the lack of spread of HIV-1 by the oral route. Infection of adherent primary monocytes with HIV-1 is dramatically suppressed in the presence of human saliva. Secretory leukocyte protease inhibitor (SLPI) was found to contribute the major portion of this antiviral activity. SLPI has potent anti-HIV-1 activity with an approximate IC_{50} of 1 μ g/ml in monocytes. SLPI is produced in the major and minor salivary glands and in other mucous producing cells of the body, but not in circulating blood cells (by current detection methods) nor in the pancreas. Additionally, SLPI is present in equivalent amounts in both healthy and HIV-1 infected individuals. The presence of SLPI in the oral cavity may be critical in the prevention of free virus infection of leukocytes found in the crevicular spaces and elsewhere in the oral cavity, thus reducing the chance of infection through the oral route. SLPI inhibits infection through interaction with the host cells. SLPI does not bind to CD4 but may interfere with an early step of virus infection, i.e. viral entry. SLPI binds with high affinity to monocytes, to a single class of receptor sites (about 7000 receptors per monocyte) with a K_d of 3.6 nM. The putative SLPI receptor was identified as a surface protein with molecular weight 58-60 kD. Recombinant SLPI variants mutated at position 72, thereby modifying antiprotease activity, still bind tightly to monocytes and retain anti-HIV-1 activity, therefore the antiprotease function of SLPI may be distinct from its anti-HIV-1 activity. The unique antiviral activity of SLPI may make it a key factor in our understanding of the early steps of HIV-1 infection of monocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00561-04 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Taste and Smell

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ryba, Nicholas	Visiting Associate	LI	NIDR
Hoon, Mark	Visiting Fellow	LI	NIDR
Siraganian, R.P.	Chief, RASTS	LI	NIDR
Wu, Youmei	Visiting Fellow	LI	NIDR

COOPERATING UNITS (if any)

Dr. R. Tirindelli, University of Parma, Italy
Dr. J. Northup, LCB, NIMH, NIH
Dr. R. F. Margolskee, Hoffman La Roche, Nutley, NJ

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.10

PROFESSIONAL:

3.10

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Molecular mechanisms of signal reception and transduction in taste and smell are being studied; particular emphasis is placed on studies of G-protein mediated signaling. Mutagenesis studies of the G-protein alpha-subunit which appears to function in bitter taste reception have revealed detailed structure-function information relevant to the mechanism of G-protein activation. A taste-tissue cDNA library has been constructed and molecular cloning studies indicate that a G-protein γ -subunit, that closely resembles the major γ -subunit of photoreceptor cones, is expressed in the circumvallate papilla. Immunolocalization of the novel G-protein γ -subunit, $\gamma 8$, that is found in developing olfactory and vomeronasal neurons has continued. The importance of C-terminal isoprenylation of G-protein γ -subunits has been investigated through functional characterization of differentially modified beta gamma-heterodimers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00609-02 LI

PERIOD COVERED

October 01, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Immune Responses in Mucosal Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: George, Anna Visiting Associate LI, NIDR
Mergenhagen, Stephan Chief, LI LI, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antigen-specific T cell responses in mucosal tissues: Mice were immunized with live cells of an enteric pathogen, and bacterial translocation into tissues was followed over time. T cells from various lymphoid organs were then cultured in vitro with soluble bacterial sonicate antigen [Ag] and Ag-specific proliferation and lymphokine-secretion were assayed. Results indicate that CD4+ cells are responsible for all three parameters studied, and that the appearance of IFN-g secreting T cells in tissues closely follows bacterial translocation from the gut. Thus, they appear in mucosal lymphoid organs [PP and MLN] before they appear in the spleen. The response also dies out faster in PP and MLN than in the spleen [4-6 wks vs 9 wks]. However, when mice are challenged with bacteria at 17 weeks, and T cells stimulated in vitro a week later, IFN-g secreting T cells are found only in the PP and MLN, and not in the spleen, indicating that while IFN-g secreting T cells might leave these sites early, quiescent 'memory' T cells might persist there. T cells in the lamina propria of the gut were then tested, and it was observed that IFN-g secreting T cells were present both at 4 and 9 weeks after immunization. However, they responded only to polyclonal stimulation, not to specific antigen. This indicates that some sort of 'end-differentiated' T cells that have lost the ability to respond to specific Ag might leave PP and MLN and home to the lamina propria, where they respond to unknown stimuli.

Role of dendritic cells [DC] in T and B cell responses: DCs grown from the bone marrow [BM] appear to be functionally 'immature' as compared to DCs isolated from peripheral lymphoid organs. When the latter are added to Tcell-Bcell microcultures, they augment antibody production by the B cells. However, addition of BM-DC to such cultures has no augmental effect. The main phenotypic difference between the two types of DCs are the levels of B7 and CD40, and experiments are in progress to determine which cytokines may convert the BM-DCs from an 'immature' to a 'mature' phenotype.

Trans-switching to IgA in IgM-transgenic mice: Mice transgenic for a rearranged IgM heavy chain, specific for phenyl-arsonate [Ar] were immunized orally with Ar-BSA in the presence of cholera toxin, and B cells from PP cells were stimulated in vitro with T cells and DC. Clones secreting anti-Ar IgA, and bearing the idiotype of the transgene were detected, indicating that in the PP of primed mice, trans-switching to IgA can occur.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00610-02 LI

PERIOD COVERED

October 01, 1994 - September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monocyte Signalling Pathways in Apoptosis and Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Um, Visiting Fellow LI, NIDR
Wahl, Sharon M. Chief, CIS LI, NIDR

COOPERATING UNITS (if any)

G. Feldman, Ph.D., FDA; Wayne Leadbetter, M.D., Orthopedic Center; Jim McCarthy, Ph.D., Univ. of Minnesota, J.M. Orenstein, M.D. and Paula Worley, GW University

LAB/BRANCH

Laboratory of Immunology

SECTION

Cellular Immunology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Monocyte apoptosis has emerged as a pivotal mechanism for controlling the outcome of inflammatory responses. Inflammatory mediators such as LPS, IL-1 β , and TNF α have been shown to prolong the life-span of human peripheral blood monocytes, and their depletion leads to apoptosis of the cells. However, the activation-dependent survival of monocytes is abolished by IL-4, an antiinflammatory cytokine or by anti-Fas antibody, suggesting that apoptosis is a fundamental mechanism for removing activated monocytes when inflammation is no longer necessary. To define the intracellular pathway leading to monocyte death, we have characterized monocyte apoptosis induced by IL-4 or anti-Fas. Activation of monocytes with cytokines or bacterial product differentially alters their susceptibility to anti-Fas and IL-4, suggesting two different apoptotic pathways. Furthermore, N-acetylcysteine (NAC), a scavenger of peroxides, inhibits apoptosis induced by anti-Fas which increases cellular levels of peroxides, but not by IL-4. In contrast, PMA, a stimulator of PKC, inhibits death mediated by IL-4, but not by anti-Fas. The role of PKC to protect monocytes from death is further supported by a PKC-specific inhibitor, calphostin C which induces apoptosis in activated monocytes. The sum of these data clearly indicates two distinct apoptotic pathways in monocytes; one involving the formation of peroxide and the other inhibited by PKC. Further unraveling of these unique pathways and their regulatory mechanism is expected to provide important information to determine potential therapeutic targets of inflammatory diseases. In this regard, experiments have been initiated to explore the role of apoptosis in chronic destructive disease and how it is regulated by immunomodulators. In additional studies to define signalling pathways for activation, rather than death, we have shown that monocyte-matrix interactions trigger jak-stat kinase signalling and augmentation of IFN γ gene induction. Inhibition of these pathways by synthetic peptide antagonists downregulates monocyte signalling and inflammatory sequelae.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00644-01 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Tyrosine Phosphatases in Cellular Signaling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Swieter, Mark	Senior Staff Fellow	LI	NIDR
Berenstein, Elsa H.	Microbiologist	LI	NIDR
Siraganian, Reuben P.	Chief, RASTS	LI	NIDR
Swaim, William D.	IRTA Fellow	LI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.8

PROFESSIONAL:

1.8

OTHER:

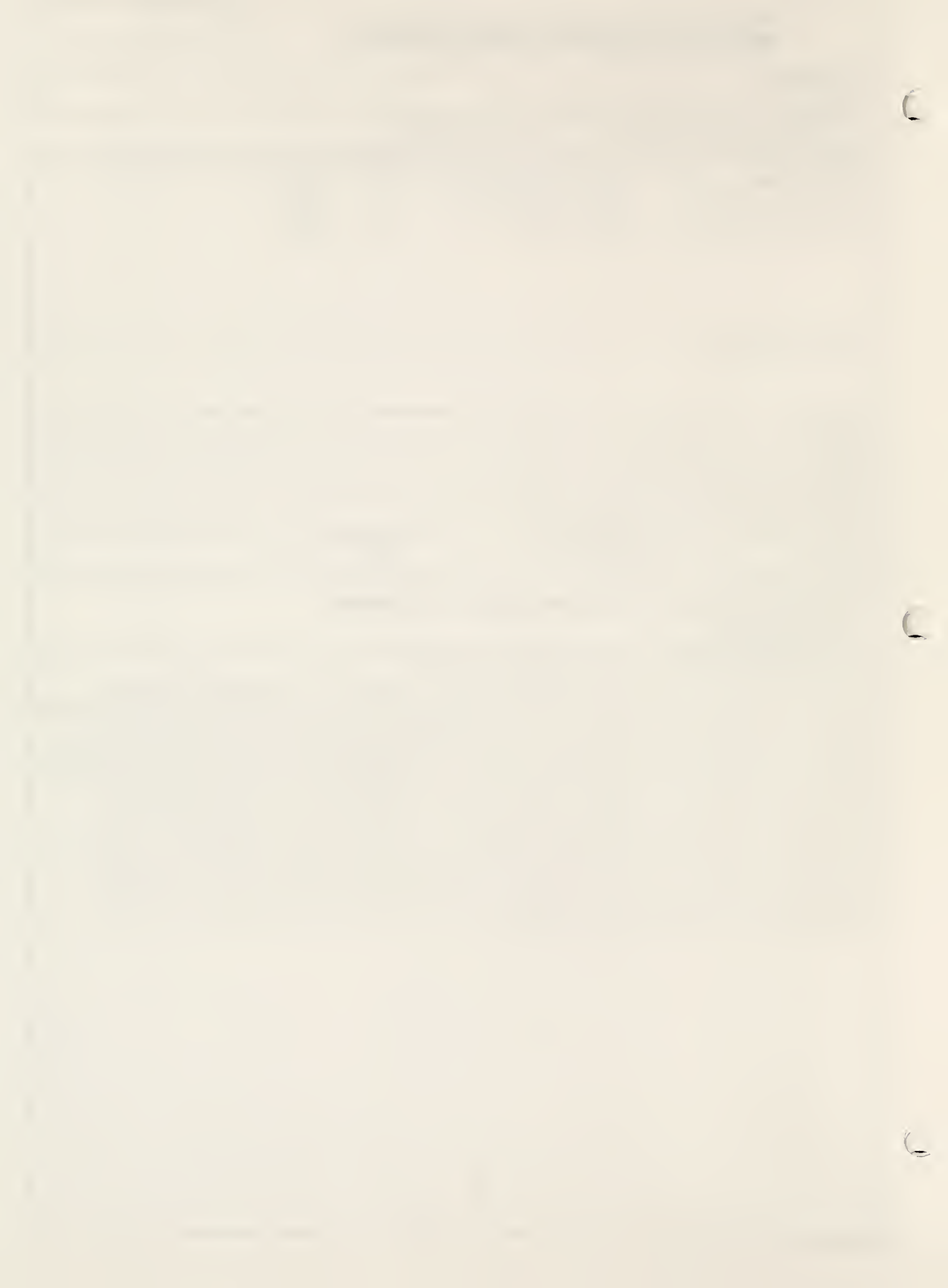
0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The roles of protein tyrosine phosphatases in immune cell function are being studied. Of particular interest are phosphatases that are involved in the cellular signaling cascade initiated at the immune response receptors. In initial work, protein tyrosine phosphatases have been identified in mast cells and basophils by biochemical and molecular cloning methods. A phosphatase activity associated with the high affinity IgE receptor in mast cells has been found. It preferentially dephosphorylates the receptor subunits but not other key signaling proteins. Therefore, it may be an important regulator of IgE receptor function. Another protein tyrosine phosphatase that seems to be restricted in its distribution to hematopoietic cells has been cloned and sequenced. It localizes to discrete subcellular compartments and becomes tyrosine phosphorylated upon IgE receptor aggregation. Thus, it too is likely to be involved in the signaling process initiated at immune response receptors.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00647-01 LI

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Adhesion Molecules in Mast Cells and Basophils Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Hamawy, Majed M.	Senior Staff Fellow	LI	NIDR
Mergenhagen, S.E.	Chief, Lab of Immunology	LI	NIDR
Sagawa, Kenji	Guest Researcher	LI	NIDR
Swieter, Mark	Senior Staff Fellow	LI	NIDR
Siraganian, Reuben	Chief, RASTS	LI	NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

Receptors and Signal Transduction Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.95

PROFESSIONAL:

1.95

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The inflammatory response is characterized by the accumulation of extracellular matrix (ECM) proteins at sites of injury and infection. Mast cells and basophils play an important role in the inflammatory response by releasing an array of mediators. Both cells have receptors, i.e. integrins, that can mediate binding to the ECM. Using the mast cell line, rat basophilic leukemia (RBL-2H3) cells, we have observed that the interaction with the ECM regulates secretion from these cells. Furthermore, such adhesion modulated FcεRI-mediated signal transduction in these cells. During the last year emphasis has been on the use of molecular biology techniques to study the intracellular mechanisms by which integrins regulate cell function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00254-18 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Microbial Antigens Associated with Specific Adherence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Cisar, John O., Research Microbiologist, LME, NIDR

Others: Hsu, S. Dana, Microbiologist, LME, NIDR

Takahashi, Yukihiko, Visiting Fellow, LME, NIDR

Sandberg, Ann L., Section Chief, LME, NIDR

COOPERATING UNITS (if any)

University of Maryland, Baltimore County; University of Texas, San Antonio; Georgetown University; University Aarhus, Denmark; FDA, Rockville

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.34

PROFESSIONAL:

0.97

OTHER:

1.37

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gram positive bacteria, primarily streptococci and actinomyces, initiate oral microbial colonization, subsequent dental plaque formation and gingival inflammation. These biological events, which represent essential steps in the development of caries and periodontal diseases, depend on specific adhesive properties, that are common to a number of oral bacteria. One such interaction is the adhesion of bacteria to the acquired pellicle, an interaction that initiates the colonization of teeth by viridans streptococci. Extensive previous studies have shown that this process involves a sialic acid reactive lectin activity and that this activity also mediates bacterial adhesions to various host cells including erythrocytes. The lectin activity of *Streptococcus gordonii* Challis has now been associated with a specific bacterial cell surface antigen. This involved the antigenic comparison of strain Challis with a spontaneous mutant that specifically lacked hemagglutinating activity. Crossed immunoelectrophoresis revealed a native antigen present in extracts of the parent strain that was absent in extracts of the nonadherent mutant. The purified antigen was composed of both protein and carbohydrate and had a fimbrial morphology based on the appearance of cells labeled with monospecific antibody prepared against the purified antigen. Significantly, Fab fragments of this antibody inhibited lectin-mediated bacterial hemagglutination, thereby associating the sialic acid binding lectin activity with a specific bacterial cell surface structure. Streptococcal cell wall polysaccharides may also play an essential role in oral microbial colonization since these molecules are recognized by lectins on other bacteria. Structural, immunochemical and genetic studies of these streptococcal polysaccharides are underway to more clearly define the determinants of immunological and lectin recognition. Insight concerning the molecular basis of various oral microbial adhesive interactions may well suggest novel approaches for the control of plaque related diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00273-17 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell-Cell Interactions Between Oral Actinomyces and Other Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kolenbrander, Paul, Research Microbiologist, LME, NIDR

Others: Andersen, Roxanna, Microbiologist, LME, NIDR

Clemans, Daniel, IRTA, LME, NIDR

Klier, Christiane, Visiting Fellow, LME, NIDR

Whittaker, Catherine, LME, NIDR

London, Jack, Research Microbiologist, LME, NIDR

Roble, Arlene, Summer IRTA, LME, NIDR

COOPERATING UNITS (if any)

Dr. P. Handley, Univ. of Manchester, England; Dr. F. Neuhaus, Northwestern Univ., Evanston, IL; Dr. H. Jenkinson, Univ. of Otago, New Zealand; Dr. A. Callaway, Mainz, Germany; E.P. Greenberg, Univ. of Iowa; R. Palmer and D. White, Univ. of Tennessee

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.79

PROFESSIONAL:

3.21

OTHER:

1.58

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of our research program is to understand the role of coaggregation in bacterial accretion of early colonizing bacteria on a clean tooth surface. The primary colonizers include actinomyces and streptococci. The 34.8-kDa lipoprotein surface-adhesin, ScaA, from *Streptococcus gordonii* PK488 is encoded as part of a 2.55 kb transcription unit consisting of three genes. This unit is a putative ATP-binding cassette operon similar to the operons encoding the binding-protein dependent transport systems of Gram-negative bacteria and the binding lipoprotein dependent transport systems of Gram-positive bacteria. ScaA probably is anchored in the cell membrane through its lipid moiety, while its receptor-recognizing binding site is exposed to the environment.

Two coaggregation-relevant genes in *Streptococcus gordonii* DL1 have been identified by transposon mutagenesis and by integrative mutagenesis. By sequencing the DNA flanking the transposon, a close relationship was found to genes involved with synthesis of lipoteichoic acids, another class of surface molecules. The sequence flanking the erythromycin insertion plasmid had little homology with other sequences in the gene bank, but these mutants have lost a 100-kDa protein from their surface. This gene has been cloned and transformants regained the COG+ phenotype and the 100-kDa protein. It is proposed that the protein is an adhesin mediating intragenetic coaggregation.

Actinomyces serovar WVA963 strain PK1259 exhibits only lactose-inhibitable coaggregation with streptococci. One of the spontaneous COG- mutants isolated excretes a 95-kDa protein that has been purified by lactose-agarose affinity beads and by binding to the partner streptococcal cells. Antiserum to the lactose-agarose bead preparation blocks coaggregation of the parent actinomyces with streptococci, suggesting that the 95-kDa protein is the adhesin mediating this coaggregation.

The long range goal of these studies, collectively, is to elucidate the molecular mechanisms responsible for bacterial colonization in the human oral ecosystem.

Professional Personnel, continued

Maro, Maria, Predoctoral IRTA, LME, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00341-14 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Sugar Transport and Metabolism in Lactic Acid and Oral Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thompson, John, Visiting Scientist, LME, NIDR

Others: Robrish, Stanley, Research Microbiologist, LME, NIDR

Bouma, Carolyn A., Senior Staff Fellow, LME, NIDR

COOPERATING UNITS (if any)

Fales, Henry, M., Lab Chief, LCB, NHLBI; Nguyen, Nga Y., Biologist, CBER, FDA; Davidson, Barrie E., Prof. Biochemistry, Univ. of Melbourne; Hillier, Alan J., Biochemist, CSIRO, Melbourne.

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Microorganisms are causative or contributory agents in the etiology of dental caries, gingivitis and related periodontal disease(s). The pathogenicity of Gram-positive streptococci and Gram-negative Fusobacteria resides in part in the capacity of these species to form a variety of organic acids and toxic sulfur-containing derivatives as end products of carbohydrate and amino acid fermentation. Elucidation of the biochemical steps and the genetic basis for regulation of these energy-yielding pathways provides the rationale for this research program. Significant accomplishments of the past year include: 1) Demonstration that the triosephosphate gene (*tpi*) of *Lactococcus lactis* is monocistronic; 2) Elucidation of the role(s) of lysine 214 and cysteine residues in activity and cytotoxicity of β -cystathionase from *Bordetella avium*; 3) Purification, cloning, sequence analysis and expression of maltose 6-phosphate:6-phosphohydrolase from *Fusobacterium mortiferum* ATCC 25557; 4) Cloning and site-directed mutagenesis of catalytically functional residues of *Tn5306*-encoded N(5)-(carboxyethyl) ornithine synthase from *L. lactis*; 5) Purification of a novel cellobiose 6-phosphate:6-phosphohydrolase from *F. mortiferum*; 6) Chemical synthesis of unique methylumbelliferyl analogs of phospho- α and phospho- β -glucosides for use as fluorogenic reporter molecules for study of the regulation of gene expression in Fusobacteria. Results from this research program have been published in four papers in peer-reviewed, international journals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00382-12 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth and Metabolism of Oral Microorganisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robrish, Stanley A., Research Microbiologist, LME, NIDR

Others: Thompson, John, Visiting Scientist, LME, NIDR

Bouma, Carolyn, Senior Staff Fellow, LME, NIDR

Donkersloot, Jacob, Research Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

Nguyen, N., Biologist, CBER, FDA

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Activity for the hydrolysis of maltose 6 phosphate [maltose 6P] has been stabilized in sonic extracts of maltose grown *Fusobacterium mortiferum* (ATCC 25557) using dithiothreitol [DTT]. All of the activity was found in a single protein with a molecular weight of 49 KD following purification in the presence of DTT and assay with an artificial alpha glucoside phosphate. The purified protein hydrolyzed authentic maltose 6P to equimolar amounts of glucose 6P and glucose confirming a phosphoenolpyruvate phosphotransferase (PTS) activity for maltose use by *F. mortiferum*. The hydrolytic activity of maltose 6P hydrolase was restricted to alpha glucoside phosphates and required Mn^{++} . An artificial alpha glucoside phosphate proved necessary for the purification of the protein and an antibody was made to the resultant 49 KD homogeneous protein with enzyme activity. Maltose 6P hydrolase activity was induced in *F. mortiferum* by growth on a variety of sugars; mostly, but not exclusively, alpha glucosides. The specific activity of the hydrolase enzyme was proportional to a hierarchy of growth sugars and the activity was exclusively in a 49 KD protein. The sequence of 32 amino acids from the NH_2 terminal end of the maltose 6P hydrolase was determined and the activity was cloned, sequenced, and expressed in *Escherichia coli*. Growth on beta glucosides induced the anaerobic use of cellobiose by washed cell suspensions of *F. mortiferum*. A 54 KD protein with activity for the hydrolysis of a beta glucoside phosphate has been purified and identified from sonic extracts of cellobiose grown cells. The properties of this beta glucoside phosphate hydrolase and its functioning in a cellobiose PTS in *F. mortiferum* are now being studied. Maltose is used by *F. necrophorum*, but by a different mechanism than that reported by us for *F. mortiferum*. The 49 KD protein for the maltose 6P hydrolase could not be demonstrated in a sonic extract of maltose grown cells of *F. necrophorum*. Two strains of *Streptococcus mutans*, reported to have a maltose PTS, had no detectable activity when sonic extracts were tested with antibody to the *F. mortiferum* maltose 6P hydrolase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00454-09 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Surface Molecules in Colonization and Biological Mimicry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: London, Jack P., Section Chief, LME, NIDR

Others: Bouma, Carolyn, Senior Staff Fellow, LME, NIDR

Kolenbrander, Paul E., Research Microbiologist, LME, NIDR

Lunsford R. Dwayne, Senior Staff Fellow, LME, NIDR

COOPERATING UNITS (if any)

Dr. A. Hand, University of Connecticut; Dr. J. Manch-Citron, University of Missouri; and
Dr. I. Weiss, Tel Aviv University, Tel Aviv, Israel

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bacterial colonization and invasion depend upon a microbe's ability to attach to a host and multiply. Oral bacteria, like other human commensals and pathogens, have evolved specific surface proteins for this purpose. Some of these molecules are associated with scaffolding structures, e.g., pili or fimbriae, and others are integral parts of the organism's outer membrane. Several adhesins on the surface of *Prevotella loescheii* have been the focus of studies in this laboratory. The gene for a 75 kDa lectin-like protein was cloned and its structure deduced from the nucleotide sequence. Translation of the adhesin mRNA occurs via a frameshifting hop in which 27 nucleotides are bypassed. Since bypasses are rarely seen among prokaryotes, it was essential to establish that the amino acid sequences prior to and immediately following the hop were the expected residues. Recent attempts to sequence this region yielded the anticipated amino acid sequence proving that a 27 nucleotide bypass occurs during translation. Elements of the "hop", e.g., the shift region, stem loop and pseudoknot are being assessed for their effect on translation of the adhesin and heterologous proteins. β -galactosidase constructs with the 228 nucleotide hop positioned in frame at the 5' end of the gene indicated that synthesis of the enzyme in *Escherichia coli* was being retarded compared to wild type production. Current studies using site-specific mutagenesis have demonstrated that regions within the stem loop and pseudoknot are absolutely essential for read-through of the adhesin mRNA. Attempts to identify and clone the gene encoding a *P. loescheii* non-lectin adhesin using degenerated probes based on the first 10 amino acids of the N-terminal sequence are continuing. The gene of a *P. loescheii* surface component believed to be a support protein was cloned and sequenced. Comparisons indicated that the deduced sequence showed extensive identity to both eukaryotic and prokaryotic enolases. The partially purified protein from which the N-terminal amino acid sequence was obtained shows enolase activity. The role of this enzyme is being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00514-06 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anthrax Toxin - A Model for Bacterial Pathogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Leppla, Stephen H., Research Chemist, LME, NIDR

Others: Keith, Jerry M., Laboratory Chief, LME, NIDR
Klimpel, Kurt R., Senior Staff Fellow, LME, NIDR
Arora, Naveen, Visiting Associate, LME, NIDR
Gordon, Valery M., Staff Fellow, LME, NIDR
Stepanov, Alexey, Visiting Fellow, LME, NIDR
Singh, Yogendra, Guest Researcher, LME, NIDR

COOPERATING UNITS (if any)

Laboratory of X-ray Crystallography, Dana-Farber Cancer Institute (R.C. Liddington)
Institute of Animal Health, Tsukuba, Japan (I. Uchida)
Laboratory of Molecular Biology, NCI, NIH (D.J. FitzGerald)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.22

PROFESSIONAL:

3.22

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure and function of bacterial protein toxins is studied to determine how toxins contribute to bacterial pathogenesis. Mammalian cells are studied to identify the subcellular targets of the toxins.

A. A collaborative project to determine the structure of anthrax toxin protective antigen (PA) by X-ray diffraction is nearing completion. Mutant PA proteins containing single cysteine residues yielded heavy metal derivatives that helped to solve the structure. Nearly all the amino acids have been located in the three-dimensional structure. The structure is being used as a guide for mutagenesis.

B. Biochemical and genetic methods are used to identify the animal cell systems involved in toxin action. Mutations are induced in cultured cells to confer resistance to bacterial toxins and fusion proteins. Characterization of these mutants helps to identify toxin receptors, activating proteases, endocytic uptake mechanisms, intracellular trafficking, and cytosolic catalytic activities.

C. Site-specific mutagenesis is used to identify functionally important amino acids in toxin proteins and in cytotoxic fusion proteins. Proteins are expressed in *Bacillus* sp., secreted to the culture medium, and purified by chromatography. Functional assays are performed in cultured cell lines. Non-toxic mutant proteins are evaluated for possible use in vaccines.

D. Toxin fusion proteins are used as a general system for delivering heterologous polypeptides into the cytosol of animal cells. The fusion proteins have residues 1-254 of anthrax toxin lethal factor attached to catalytic domains from several different toxins. When combined with PA, these fusion proteins are highly toxic to mammalian cells because they are efficiently translocated to the cytosol. Fusions of polypeptides from transcriptional activators, regulatory substances, etc. are designed that will have a therapeutic action when delivered to cytosol of animal cells.

Professional Personnel, continued

Jacobs, Myra F.

Visiting Fellow

LME, NIDR

Cooperating Units, continued

Department of Microbiology and Molecular Genetics, Harvard Medical School (R.J. Collier)

Division of Bacterial Products, CBER, FDA (J. Halpern)

Howard Hughes Medical Institute, Ann Arbor, Michigan (R. Kaufman, A. Rehemtulla)

Laboratory of Biochemistry and Metabolism, NIDDK, NIH (B. Sauer)

Developmental Therapeutics Program, DCT, NCI (E. Sausville)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00518-06 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Detoxified Pertussis Toxin for Acellular Whooping Cough Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Keith, Jerry M. Laboratory Chief, LME, NIDR

Others: Merkel, Tod, Staff Fellow, LME, NIDR

Barros, Cassia, Visiting Fellow, LME, NIDR

COOPERATING UNITS (if any)

NIH, Tokyo Japan (H. Sato); Washington University, St. Louis, MO (R. Curtiss III); University of Missouri, Columbia MO (C. Parker); U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, MD (D.R. Brown).

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.87

PROFESSIONAL:

1.87

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Whooping cough is caused by an infection of the respiratory tract with *Bordetella pertussis* bacteria. This disease is effectively controlled by the current vaccine which consists of killed whole *B. pertussis* cells. Though efficacious, the present vaccine produces unacceptable side effects. The major protective antigen in whooping cough vaccines is pertussis toxin. Chemically "inactivated" pertussis toxin vaccines have been produced with reduced side effects and reasonable efficacy, however, residual activity may exist. Using site-specific DNA mutagenesis, we modified *E. coli* subclones of the pertussis toxin SI subunit and then used these constructs to replace the chromosomal copy of the toxin gene in *B. pertussis* strain 3779. The resulting new strain produces a fully genetically detoxified form of pertussis toxin which is strongly immunoprotective and can be used as a vaccine antigen without chemical inactivation. In a recently completed NIAID-supported clinical trial in Sweden and Italy, pertussis toxin emerged as an essential component of any new whooping cough vaccine. One of the major acellular pertussis products used in this trial was a mutant version of pertussis toxin that was developed from basic research generated through this intramural research project. Molecular studies are currently underway in our laboratory to develop high yield *B. pertussis* strains to enhance expression of pertussis toxin for use in acellular and conjugate vaccine manufacture.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00557-04 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Specific Interactions of Bacteria with Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Sandberg, Ann, Section Chief, LME, NIDR

Others: Sutphin, Michelle, Biologist, LME, NIDR
Ruhl, Stefan, Visiting Associate, LME, NIDR
Cisar, John O., Research Microbiologist, LME, NIDR
Takahashi, Yukihiro, Visiting Fellow, LME, NIDR
Yoon, Jeong-Weon, Special Volunteer, LME, NIDR

COOPERATING UNITS (if any)

Dr. Mike Eckhaus, VRP, NCRR, NIH
Jennie Owens, VRP, NCRR, NIH

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Microbial Receptors and Pathogenesis Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.08

PROFESSIONAL:

1.18

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Further insights into the molecular bases of the lectin-mediated adhesion of oral bacteria to polymorphonuclear leukocytes (PMNs) and the biological implications of these interactions have been acquired. Two PMN glycoprotein receptors, both of which were recognized by the sialic acid reactive lectins of oral streptococci as well as the Gal/GalNAc reactive lectins of actinomyces, have been unequivocally identified. The major receptor is leukosialin (CD43). This glycoconjugate was initially detected by binding of Gal/GalNAc or sialic acid reactive plant lectins and, subsequently, by binding of the actinomyces and streptococci to a 130 kDa band on nitrocellulose transfers of PMN and differentiated HL60 cell extracts separated by SDS-PAGE. Binding of the actinomyces and plant lectins with similar specificities required exposure of the saccharide receptors by sialidase. The 130kDa band was the only glycoprotein detected by an anti-leukosialin antibody. Conclusive evidence of the receptor activity of this glycoconjugate was obtained by bacterial binding to the 130kDa band on transfers of PMN or HL60 cell extracts that were immunoprecipitated with the anti-leukosialin antibody and the immunoprecipitates subjected to SDS-PAGE. In addition, all of the previously described plant lectins recognized a 200 kDa band that was identified as the leukocyte common antigen (CD45). Both bacteria also bound to this glycoconjugate following its concentration from cell extracts by immunoprecipitation, with sialidase requirements identical to those described above. The specificities of all reactions were verified by saccharide inhibition and the failure of mutants or strains lacking the lectin activities to bind to the phagocytic cells or to the glycoconjugates on nitrocellulose transfers. Lectin-dependent interactions of the streptococci with PMNs had major implications for the initiation of endocarditis. A number of oral *Streptococcus gordonii* strains activated PMNs and were ingested by these phagocytic cells but only two were susceptible to killing. Of major interest was the finding that the strains that resisted lectin-mediated killing by PMNs produced severe endocarditis in a rat model system. In marked contrast, those strains that were killed by the phagocytic cells failed to induce endocarditis. Moreover, the production of endocarditis did not correlate with a number of previously suggested parameters. Thus, resistance to lectin-mediated killing of these bacteria by the phagocytic cells is a determinant of virulence for the initiation of endocarditis by this particular group of streptococci.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00571-03 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Genetic Competence in Oral Streptococci

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lunsford, R. Dwayne, Senior Staff Fellow, LME, NIDR

Others: London, Jack P., Research Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Clinical Microbiology

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project was initiated specifically to: I) utilize the competence system for natural genetic transformation as a model for global genetic regulation in the oral streptococci, and II) determine what role natural genetic transformation may play in the horizontal transfer of genetic information within the streptococcal compartment of the oral microbiota. Accomplishments during the covered period were:

- 1) Isolation and characterization of a cell surface DNA receptor.
- 2) Purification of HSGo, an *S. gordonii* chromatin-like protein.
- 3) Isolation of a putative CF operon.
- 4) Development of a Tn 4001 delivery system for oral streptococci.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00601-03 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV-targeted cytotoxic proteins derived from anthrax toxin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Leppla, Stephen H., Research Chemist, LME, NIDR

Others: Klimpel, Kurt R., Senior Staff Fellow, LME, NIDR

Gu, Mi-Li, IRTA Fellow, LME, NIDR

Teixeira, Avelino V., Visiting Fellow, LME, NIDR

Arora, Naveen, Visiting Associate, LME, NIDR

Gordon, Valery M., Staff Fellow, LME, NIDR

Keith, Jerry M., Laboratory Chief, LME, NIDR

COOPERATING UNITS (if any)

Division of Antivirals, FDA (M. Ussery)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.69

PROFESSIONAL:

3.69

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Unique features of anthrax toxin are being exploited to make novel, cell-specific cytotoxins for HIV-1 infected cells. The three separate strategies described below for killing cells utilize highly toxic fusion proteins in which the amino-terminal portion of anthrax toxin lethal factor (LF) is genetically fused to the ADP-ribosylation domain of *Pseudomonas* exotoxin A (PE). Delivery of these LF-PE fusion proteins to the cytosol of cells requires the prior binding and proteolytic activation of the protective antigen (PA) component of the toxin. Three approaches to targeting cells are being used:

1. The site in PA which must be proteolytically cleaved is replaced by consensus sequences recognized by HIV-1 protease, to make a mutant PA that will be activated only in HIV-1-infected cells. Many of the mutant PA proteins produced were cleaved by HIV-1 protease in vitro. Surprisingly, several were toxic with LF for normal, non-infected cells, showing that endogenous proteases cleave within the newly added sequences. Additional target sequences will be constructed to find ones cleaved by the HIV-1 protease but not by endogenous cellular proteases.
2. CD4 and IL-2 are fused through a polypeptide linker to the carboxyl terminus of PA that has been altered to remove its ability to bind to its own receptor, either by truncation of the carboxyl terminus or mutagenesis of specific residues involved in receptor binding.
3. The gene encoding PA is transfected into several different mammalian cells to test the feasibility of sensitizing cells to LF fusion proteins by intracellular production of PA.

The role of furin in processing HIV-1 gp160 is studied using hamster cells that are either normal, furin-deficient, or expressing furin from cDNA vector. Processing of gp160 is assessed biochemically and viral replication is tested by co-cultivation with virus-susceptible T cells.

Professional Personnel, continued

Jacobs, Myra F.	Visiting Fellow	LME, NIDR
McDonald, Furman S.	HHMI-NIH Research Scholar	LME, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00604-02 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics and Biochemistry of Penitol Metabolism in Oral Lactic Acid Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bouma, Carolyn, Senior Staff Fellow, LME, NIDR

Others: London, Jack P., Section Chief, LME, NIDR

Hall, Jessica, Special Volunteer, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.40

PROFESSIONAL:

0.15

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Lactobacillus casei, an oral bacterium prevalent in carious lesions, is one of only two lactic acid species capable of utilizing the pentitols xylitol (Xtl) and ribitol (Rtl) as growth substrates. Such polyols occur naturally in plants and are used as artificial sweeteners in sugar-free products. While cariogenic streptococci cannot metabolize these polyols, *L. casei* and *Enterococcus avium* are capable of producing acid from them. Studying the enzymes involved in metabolism of Rtl and Xtl is therefore pertinent to oral health. *L. casei* transports Rtl and Xtl via a specific phosphotransferase system (PTS). The Rtl and Xtl pathways each comprise a membrane-bound permease, a soluble III-ribitol or III-xylitol, and a pentitol-5-phosphate dehydrogenase. It is our goal to characterize the structure and function of the protein components of the Rtl and Xtl PTS pathways, and to study their underlying mechanisms of genetic regulation. Our approaches focus on Rtl-5-P dehydrogenase (RtlA), the *rtl* operon repressor protein (RtlR), and III-xylitol. *RtlA* was cloned from and *L. casei* DNA library by hybridization and immunological screening. *RtlA* shares sequence identity with several NADH-dependent dehydrogenases, but is not related to the functionally similar dehydrogenases of the hexitol PTS of enteric bacteria. Another gene, designated *rtlR*, was identified and potentially encodes a repressor protein. *RtlR* shares identity with members of the DeoR family of transcriptional repressors, including regulators of other PTS operons such as the *Streptococcus mutans* and *Lactococcus lactis* lactose PTS. The genes encoding the Rtl permease and III-ribitol were not present within the cloned *L. casei* DNA fragment carrying *rtlA* and *rtlR*. The transcriptional initiation sites of *rtlA* and *rtlR* were identified and permitted the localization of the promoters for these genes. A bacteriophage clone isolated from an *L. casei* library directs the synthesis of a polypeptide which cross-reacts with anti-III-xylitol antisera. The genetic analysis of this clone is in progress. We propose to investigate the regulation of the *rtl* operon and to explore the relatedness of the *E. avium* and *L. casei* Rtl and Xtl PTS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00607-02 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibiotic Resistance among Streptococci

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Donkersloot, Jacob A., Research Microbiologist, LME, NIDR

Others: Pikis, Andreas, Staff Fellow, LME, NIDR

Harr, Robert J., Biolaboratory Technician, LME, NIDR

Keith, Jerry M., Laboratory Chief, LME, NIDR

COOPERATING UNITS (if any)

William J. Rodriguez, Dept. of Infectious Disease, Children's National Medical Center,
Washington, D.C. 20010

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.45

PROFESSIONAL:

1.7

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Streptococcus pneumoniae is a significant cause of morbidity and mortality in pediatric, geriatric, and immunocompromised populations. Until the mid-sixties, this pathogen was uniformly susceptible to penicillin, but since then the incidence of resistance to this antibiotic (as well as many others) has increased to such an extent that infections due to penicillin-resistant pneumococci have become a major threat in many countries. With regard to the USA, a nationwide survey showed that 5% of the pneumococci isolated in hospitals during the period 1979-'87 were intermediately resistant to penicillin (minimum inhibitory concentration (MIC) = 0.1-1 mg/L), and only 0.02% highly resistant (MIC > 1 mg/L). Under the aegis of a Children's Hospital (Washington, D.C.) - NIDR clinical research training program, a pilot project was initiated to assess the prevalence of penicillin resistance among pneumococci isolated from patients attending this hospital. The one-year survey (from June 1992 through May 1993) showed that 8.3% of the 108 strains isolated were intermediately resistant to penicillin and 4.6% were highly resistant. Moreover, at least 40% of the penicillin-resistant strains were also resistant to frequently used oral and parenteral cephalosporins (cefactor, cefixime, cefotaxime, cefpodoxime, cefuroxime, cephalexin, and loracarbef) and carbapenems (imipenem and meropenem). Also of great concern was the finding that all isolates were resistant to trimethoprim/sulfamethoxazole, a drug that is commonly used to treat infections in ambulatory patients. As a result of these findings, a study to define, at the sequence level, the molecular epidemiology and mechanism of trimethoprim resistance in *S. pneumoniae* was initiated. Initially, the trimethoprim-resistance determinant of several clinical isolates will be cloned, sequenced, and analyzed. Identification of specific residues responsible for resistance will also require the cloning and sequencing of the trimethoprim target protein from a sensitive strain. This cloning can be done by the PCR with primers based on sequences that are conserved in the resistant isolates. Alternatively, a library of the sensitive strain can be screened with gene probes derived from one or more resistant isolates. The information obtained from the comparative sequence analysis will be combined with modeling of the TMP-target protein to design novel antibiotics that will be effective against trimethoprim-resistant streptococci.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00612-02 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stimulation of Cellular Immunity with Anthrax Lethal Toxin-Antigen Fusions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Klimpel, Kurt, Senior Staff Fellow, LME, NIDR

Others: Arora, Naveen, Visiting Associate, LME, NIDR
Leppla, Stephen, Research Chemist, LME, NIDR
Jacobs, Myra, Visiting Associate, LME, NIDR
Keith, Jerry M., Laboratory Chief, LME, NIDR

COOPERATING UNITS (if any)

Berzofsky, Jay, NCI; Goletz, Terry, NCI

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccine Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.72

PROFESSIONAL:

0.72

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Bacillus anthracis secretes two toxins into the extracellular medium during growth. The two toxins consist of three distinct proteins which combine in a pairwise fashion. Protective antigen (PA) can combine with lethal factor (LF) or edema factor (EF). PA combined with LF makes lethal toxin while PA combined with EF makes edema toxin. Part of each toxin is directly transported to the cytosol of living cells where it exerts its effect (see Arora, N., K.R. Klimpel, Y. Singh, and S.H. Leppla, 1992, J Biol Chem 267:15542-15548). We propose to take advantage of the efficient delivery of proteins to the cytosol to intracellularly inoculate living cells. Nearly all cell types have the ability to process proteins found in the cytosol and combine them with MHC class I molecules. The combination of the processed protein with the MHC class I molecule presented on the surface of the living cell results in the stimulation of a population of T-cells which recognize the processed protein antigen. Once stimulated, this population of T-cells can expand and become primed to rapidly respond to and eliminate cells which bear an identical combination of MHC class I and processed antigen. By making fusions between LF and different polypeptide antigens we may be able to vaccinate a host against many pathogens. Our initial work will focus on priming a response against several known antigenic proteins expressed by HIV-1, including gp120, gp41 p24, Nef, Tat and Rev.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-DE00642-01 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Techniques for the Genetic Manipulation of Oral Anaerobic Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bouma, Carolyn Senior Staff Fellow, LME, NIDR

Others: Thompson, John, Visiting Scientist, LME, NIDR

Robrish, Stanley, Research Microbiologist, LME, NIDR

COOPERATING UNITS (if any)

Blattner, Frederick R., Director, E. coli Genome Project, University of Wisconsin-Madison, Madison, WI; Plunkett, Guy, Laboratory of Genetics, University of Wisconsin-Madison, Madison, WI

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD

TOTAL STAFF YEARS:

0.9

PROFESSIONAL:

0.9

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The role of Gram-negative anaerobic microorganisms as causative or contributory agents in a variety of human diseases is well-documented. For example, the oral anaerobe *Fusobacterium nucleatum*, in addition to being the most abundant bacterium in periodontal pockets, is frequently involved in abscesses of the periodontium, head and neck infections, chronic sinusitis, otitis media and pleuropulmonary infections. Despite their obvious role in disease, the pathogenic mechanisms of *Fusobacterium spp.* are virtually unknown. For this reason we propose to develop the molecular genetic techniques necessary for the identification of virulence factors associated with oral anaerobes, particularly members of the genus *Fusobacterium*. In order to develop such a system, genes encoding metabolic traits must be characterized and incorporated as selectable markers in a plasmid- or transposon-based gene transfer system that is functional in *Fusobacterium spp.* Except for preliminary studies conducted in this laboratory, the aforementioned prerequisites for genetic analysis have yet to be met for *Fusobacterium spp.* We have succeeded in introducing broad host-range plasmids into *F. mortiferum* ATCC 25557 via conjugation. In addition, we have identified two systems unique to *F. mortiferum* (namely, the maltose and cellobiose phosphotransferase systems) which are eminently suitable as selectable metabolic traits for gene transfer studies. Utilization of a metabolic system as a marker avoids the limitations imposed on selection in strains that are naturally resistant to multiple antibiotics. Significant accomplishments include the purification of maltose-6P:6-phosphohydrolase (MalH) from *F. mortiferum* ATCC 25557; the cloning, expression in *Escherichia coli*, and sequence analysis of MalH; and the purification of cellobiose-6P:6-phosphohydrolase from *F. mortiferum* ATCC 25557; and organization of current transposon mutagenesis and conjugative systems for use in *Fusobacterium spp.* Elucidation of the biochemical steps and the mechanism of genetic regulation of the maltose and cellobiose phosphotransferase systems are prerequisite to their use as markers in a gene transfer system for oral anaerobes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00648-01 LME

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Analysis of *Fusobacterium* Pathogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Keith, Jerry M. Laboratory Chief, LME, NIDR

Others: Gentry-Weeks, Claudia, Senior Staff Fellow, LME, NIDR

Diglisic, Gordana, Visiting Fellow, LME, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Ecology

SECTION

Bacterial Toxins and Vaccines Section

INSTITUTE AND LOCATION

National Institute of Dental Research, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.95

PROFESSIONAL:

1.95

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fusobacterium necrophorum was tested for the ability to survive and multiply in mouse peritoneal macrophages. *F. necrophorum* (5×10^8) was injected intraperitoneally into mice and following an infection period of 4 hours, the peritoneal macrophages were harvested, exposed to gentamycin, and maintained in tissue culture. Macrophages were lysed at 4, 24, 48, and 72 hours postinfection, and lysates were plated on agar medium and incubated anaerobically to recover viable bacteria. *Escherichia coli* LE392, a nonvirulent laboratory strain, served as a control in these experiments. *F. necrophorum* survived and multiplied in mouse peritoneal macrophages for at least 72 hours at a level of 10^5 fold higher than the control. *E. coli* LE392 survived for 24 hours in the mouse peritoneal macrophages but the number of bacteria dropped precipitously by 48 hours. Electron micrographs of the *F. necrophorum*-containing macrophages revealed that *F. necrophorum* were initially enclosed within a membrane-bound phagolysosome, but by 24 hours postinfection, the phagolysosome membrane was dissolved, and by 48 hours the macrophages were being destroyed and *F. necrophorum* was released. A gene library of *F. necrophorum* DNA was constructed in *E. coli* LE392 in order to identify and characterize the gene(s) involved in survival of *F. necrophorum* in mouse peritoneal macrophages. One thousand recombinant *E. coli* clones were tested for their ability to survive in mouse peritoneal macrophages. Two *E. coli* clones were identified which were able to survive for 72 hours in mouse peritoneal macrophages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01DE00423-10 LOM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cloning, Expression and Characterization of Human Pancreatic Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI:	Lan, Michael S.	Senior Staff Fellow	LOM, NIDR
Others:	Borovitskaya, Anna	Visiting Fellow	LOM, NIDR
	DeSilva, Mark G.	Visiting Associate	LOM, NIDR
	Donadel, Giulia	Visiting Associate	LOM, NIDR
	Li, Qing	Visiting Fellow	LOM, NIDR
	Lu, Jia	Visiting Fellow	LOM, NIDR
	VanderVegt, F.P.	IRTA	LOM, NIDR
	Xie, Hong	Visiting Fellow	LOM, NIDR

COOPERATING UNITS (if any)

Dr. Noel Maclaren, University of Florida, Gainesville, FL; Dr. R.D.G. Leslie, St. Bartholomews Hospital, UK

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

8.45

PROFESSIONAL:

7.7

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

X (a) Human Subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two genes, IA-1 and IA-2, are the focus of our study. We determined the promoter region of IA-1 gene by transient transfection assay. These experiments showed that a 506 bp upstream sequence was sufficient for maximal expression of a reporter gene. Multiple known regulatory elements were found within this region including three E boxes and a clustered Sp-1 site. The promoter region of the IA-1 gene was located from -111 bp to +26 bp and displayed cell-specificity. Several promoter-binding nuclear factors specific for pituitary tumor and insulinoma cells were identified suggesting that they might be involved in the cell-specific expression of IA-1. IA-2 is a 105,874 kDa transmembrane protein that belongs to the protein tyrosine phosphatase family. We tested the reactivity of sera from patients with IDDM using full-length cDNA clone of IA-2 in a rabbit reticulocyte transcription/translation system. One hundred coded sera were tested, 50 from patients with newly diagnosed IDDM and 50 from age-matched normal controls. Sixty-six percent of the sera from diabetics, but none of the sera from controls, reacted with IA-2. A prospective study of IA-2 autoantigen (12 years duration) in 33 non-diabetic identical twins of IDDM probands and 38 controls showed that 11 twins developed IDDM. Ten of 11 prediabetic twins developed autoantibodies to IA-2 many years before the onset of IDDM, while only one of the 22 remaining non-diabetic twins developed autoantibodies to IA-2. Autoantibodies to IA-2 were detected in prediabetic sera more frequently than in non-diabetics sera (51/56 vs 1/70) ($p < 0.001$). The diagnostic value for newly-onset IDDM and the high predictive value from the twin study suggest that IA-2 is a major autoantigen for IDDM.

Professional Personnel, continued

Zhang, Baowei	Visiting Fellow	LOM, NIDR
Notkins, Abner L.	Medical Director	LOM, NIDR
Solomon, Janice	Editorial Assistant	LOM, NIDR
Trado, Dorothy	Secretary	LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 DE00471-08 LOM
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PERIOD COVERED October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transgenic Mice as Models for the Study of HIV-1 Pathogenesis
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PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)			
PI:	De, Swapan	Senior Staff Fellow	LOM, NIDR
Others:	Marinos, Nancy	Bio. Lab Technician	LOM, NIDR
	Wohlenberg, C.	Microbiologist	LOM, NIDR
	Monell-Torrens, E.	Bio. Lab Technician	LOM, NIDR
	Notkins, Abner L.	Medical Director	LOM, NIDR
	Kopp, Jeffrey	Sen. Research Investigator	LOM, NIDR
	Mozes, Miklos	Special Volunteer	LOM, NIDR

COOPERATING UNITS (if any) Chi Chao Chan, NEI; J. Kopp, KDS, NIDDK; W. Kajiyama, KDS, NIDDK; Miklos Mozes, KDS, NIDDK
--

LAB/BRANCH Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.32	PROFESSIONAL: 2.25	OTHER: 3.07
----------------------------	-----------------------	----------------

CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		

Transgenic mice provide a unique model system to investigate the molecular and cellular mechanisms of HIV pathogenesis. Studies in HIV-transgenic mice from several laboratories, including our own, have helped to clarify the role of HIV-1 gene products in inducing disease independent of opportunistic infection. In the previous work carried out in the LOM a genetically engineered HIV-1 proviral genome containing the env, tat, rev, nef, vpr, vip and vpu genes under the control of the HIV-1 long terminal repeat (LTR) was introduced into the mouse genome by pronuclear microinjection. Transgenic mice express viral mRNA and envelope glycoprotein in various tissues. They develop some of the manifestations of AIDS such as renal disease, hyperproliferative skin disorders and cataract. Homozygous mice develop a diffuse epidermal hyperplasia, a syndrome of growth failure, cachexia with lymphoproliferation and usually die before 30 days of age. These results implied a role for one or more of the HIV-1 gene products encoded by the viral transgene in the etiology of these pathologies.

In the past year we have investigated the role of HIV-1 protein in skin transplant rejection, and have shown that transgenic skin expressing viral proteins is rejected by a congenic recipient animal but not by another transgenic mouse. These data suggest that the transgenic mice become tolerant to viral proteins expressed in the skin. We have characterized the eye disease seen in d1443 mice, and found gp 120 expression in the lens fiber cells, suggesting that expression in these cells results in a disordered lens matrix and ultimately to nuclear cataracts. We have observed that mice homozygous for the HIV transgene maintain normal intrauterine growth throughout the pregnancy and are born weighing the same as the heterozygous and normal nontransgenic mice. Preliminary studies showed that some pregnancy related hormones can regulate the viral gene expression in these transgenic mice. Estrogen and progesterone can enhance and human chorionic gonadotropin can inhibit the levels of viral gene expression in a tissue specific manner. We have examined the effect of beta human chorionic gonadotropin on the wasting syndrome seen in the homozygous HIV-1 transgenic mouse, and found that this agent reduces transgene expression in muscle and improves survival in a dramatic fashion. In further characterizing the nephropathy, we have extended our studies of kidney disease to mice transgenic for an identical construct but lacking the nef gene, and have localized HIV-1 gene and protein expression to the glomerular epithelium. These cells are notable for being a major site of pathology in both the transgenic mice and human patients with HIV associated nephropathy, suggesting that they may be key target cells for HIV-1 mediated pathology.

Professional Personnel, continued

Kajiyama, Wataru	Visiting Associate	LOM, NIDR
Trado, Dorothy	Secretary	LOM, NIDR
Eloise Mange	Editorial Assistant	LOM, NIDR

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 DE00619-02 LOM
PERIOD COVERED October 1, 1994 to September 30, 1995		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Intracellular Immunization		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Zhou, Paul	Senior Staff Fellow LOM, NIDR
Others:	Devadas, Krishnar	Visiting Associate LOM, NIDR
	Tewari, Deepanker	Visiting Fellow LOM, NIDR
	Notkins, Abner L.	Medical Director LOM, NIDR
	Trado, Dorothy	Secretary LOM, NIDR
	Marinos, Nancy	Bio. Lab Technician LOM, NIDR
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Oral Medicine		
SECTION		
INSTITUTE AND LOCATION NIDR, NIH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.15	PROFESSIONAL: 2.65	OTHER: .50
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Antibodies play a crucial role in humoral immunity. Since physiologically, they are either expressed on cell surface or secreted into body fluid, they work exclusively extracellularly. Therefore, they play a limited role in defense against many intracellular pathogens. Recently, several anchor domains responsible for intracellular localization of a given protein have been identified. As a result, it is becoming possible to target genetically engineered molecules to specific intracellular compartments. The purpose of this project is to determine whether antibody genes can be directed and expressed in appropriate intracellular compartments and, if so, whether they will effect physiologic and pathologic processes. The model system being developed involves the expression of anti-HIV-1 antibody genes in human CD4 ⁺ T cell lines. During the past year, we have progressed in several areas of this project. 1) We have isolated and sequenced the full-length cDNA's containing heavy and light chain antibody genes specific for HIV-1 protease from a hamster B hybridoma. Single-chain Fv (scFv) and scFv/C _{kappa} gene constructs specific for HIV-1 protease have been made. 2) We have expressed the scFv specific for HIV-1 gp41 in bacteria and found that the scFv can specifically bind to antigen(s). 3) The anti-HIV-1 gp41 scFv's with or without ER and TGN anchor domains have been expressed in COS and human Jurkat T cell lines. The results showed that without the ER- or TGN-anchor the scFv's are secreted from cells and with ER- or TGN-anchor the scFv's are captured inside cells. The kinetic study indicates that the half life of the scFv-ER is over 24 hours and the half life of the scFv-TGN is about 9 hours. We are now testing the effect of these scFv's on the infectivity of HIV-1. 4) We have generated transgenic mice containing scFv-ER and scFv-TGN specific for HIV-1 gp41. We will mate them to our HIV-1 transgenics to test the <u>in vivo</u> effect of the intracellular scFv.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE00620-02 LOM

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen-Binding B Cells and Polyreactive Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Chen, George	Senior Staff Fellow	LOM, NIDR
Others:	Shimizu, Fumio	Guest Researcher	LOM, NIDR
	Deng, Jack	IRTA	LOM, NIDR
	Notkins, Abner L.	Medical Director	LOM, NIDR
	Wheeler, James	Biologist	LOM, NIDR
	Monell-Torrens, E.	Bio. Lab Technician	LOM, NIDR
	Trado, Dorothy	Secretary	LOM, NIDR
	Cheung, Sau C.	Staff Fellow	LOM, NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Oral Medicine

SECTION

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.71

PROFESSIONAL:

3.11

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polyreactive antibodies (Ab) are naturally occurring Abs, primarily of the IgM type, and are capable of reacting with a wide variety of antigens (Ags). As the biological function of polyreactive Abs, polyreactive Ab-producing B cells, and the molecular structure of the paratope responsible for multiple Ag binding are still unclear, the objectives of this study are: 1) to find a cellular marker which can be used to identify human B cells making polyreactive Abs; 2) to study the function(s) of polyreactive Ab-producing cells; and 3) to define molecularly the paratope structure responsible for multiple Ag-binding of polyreactive Ab.

Ag-binding and non-Ag-binding B cells were isolated by FACStar^{plus} and analyzed for their ability to make polyreactive Abs. Four to six times more cells making polyreactive Abs were found in the B cell subset that bound Ags than in the B cell subset that did not bind Ags. FACS analysis revealed that cell lines making polyreactive Abs bound a variety of Ags, whereas cell lines making monoreactive Abs bound only a single Ag. Both CD5⁺ and CD5⁻ Ag-binding B cells made polyreactive Abs, but the frequency was slightly higher in the CD5⁺ Ag-binding (85%) as compared to the CD5⁻ Ag-binding (50%) population. Comparison of Ag-binding and non-Ag-binding CD5⁺ B cells showed that approximately 86% of the former, but only 15% of the latter, made polyreactive Abs. FACS analysis further revealed that few Ag-binding B cells express B7 molecules (i.e. B7-1 and B7-2). Examination of the frequency of the polyreactive Ab-producing precursor cells in the B7⁺/non-Ag-binding and the B7/Ag-binding B cell populations showed that approximately ten times more polyreactive Ab-producing precursor cells were found in the latter than in the former. No upregulation of B7-1 and/or B7-2 expressions were observed on B cells up to 48 hrs after incubation with thyroglobulin (Tg) and β -gal which these cells bound with low affinity. In contrast, a strong upregulation of B7-1 and B7-2 expression were detected after 24 hrs incubation with anti-human Ig xenoantibodies which B cells bound with high affinity. In other experiments ¹²⁵I-Ag was incubated with a human polyreactive Ab producing hybridoma cell line (mAb63), and the conversion of TCA-insoluble ¹²⁵I-Ag protein into TCA-soluble ¹²⁵I-Ag peptides was determined. We found that the polyreactive cell line was able to process ¹²⁵I-IgGFc, -Tg and -insulin, although not as efficiently as it processed ¹²⁵I-anti-human Ig xenoantibodies which bound with high affinity. In contrast to polyreactive hybridomas, monoreactive hybridomas processed only the Ag to which the surface Ab reacted. Our results suggest: 1) Ag-binding can be used as a marker to identify polyreactive Ab-producing precursor B cells; 2) polyreactive Ab-producing precursor cells are B7 negative cells; 3) low affinity Ag binding does not lead to the upregulation of B7 molecules; and 4) polyreactive Ab-producing cells can process multiple Ags. The study on the molecular structure of the polyreactive Ab paratope was only recently initiated and is progressing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00031-27 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Design and Computer Interfacing of Neurophysiological Instrumentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Brown, Frederick J. Electronic Engineer (Instru) NAB NIDR

Others:

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These projects involve the design and construction of electronic electromechanical instrumentation to be used in neurophysiological, physiological and behavioral research. Projects also include the interfacing of these and other instruments to laboratory computers. Electronic circuit design, microcomputers, and assembly language programming may be used in these instruments or interfaces.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00132-21 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacologic Modulation of Neuroendocrine Responses to Stress and Inflammation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dionne, Raymond A.	Chief, CPU	NAB NIDR
Others: Gordon, Sharon	NRSA Fellow	NAB NIDR
Dubner, Ronald	Chief, NAB	NAB NIDR
Brahim, Jaime	Oral Surgeon	CIPCB NIDR

COOPERATING UNITS (if any)

McCullough, Linda	Staff Nurse	CC Nursing
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LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.97

PROFESSIONAL:

0.97

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are 1) to evaluate the neuroendocrine responses to surgical stress and inflammation, 2) to determine the analgesic and anti-inflammatory effects of prototype and novel drugs which alter either the synthesis or the receptor activation of neuroendocrine mediators, and 3) to evaluate the clinical utility of these novel drugs in controlled clinical trials.

Previous work on the physiologic function of plasma beta-endorphin and regulation of its release has provided evidence of enhanced release by a variety of stressors, including clinical pain following oral surgery and chest pain in patients with coronary artery disease. A current clinical study is attempting to measure inflammatory mediators released following surgery in order to determine the relationship between local levels of inflammatory mediators, clinical reports of pain, and their modulation by local infusion of prototypic drugs into the site of inflammation. Subjects undergoing the removal of an impacted third molar have a microdialysis fiber placed under the mucoperiosteal flap which is then slowly perfused to allow diffusion of mediators from the extracellular fluid into the perfusion fluid. Prostaglandin E2 is used as a marker of inflammatory mediators and increases postoperatively in the microdialysate coincident with the onset of acute postoperative pain. Systemic administration of ketorolac decreases both pain and PGE2 levels, suggesting a functional relationship between NSAID analgesia and levels of locally released PGE2. A recent study demonstrated that administration of a very low dose of ketorolac (1 mg) administered directly into the extraction site at pain onset resulted in analgesia comparable to systemic administration of a normal therapeutic dose of 30 mg. Preliminary results suggest that peripheral administration of the NSAID is temporally related to decreased PGE2 levels at the extraction site. These data support a peripheral site of action of NSAIDs and provide a rationale for evaluating the effects of other peripherally administered drugs on pain and inflammatory mediators.

Increasing evidence suggests that the nociceptive afferent barrage which can occur during a surgical procedure can activate central processes leading to an increased perception of clinical pain long after the nociceptive input is removed. This hypothesis was evaluated in the oral surgery model by randomly allocating local anesthesia or placebo anesthesia prior to the surgical removal of third molars with general anesthesia. Plasma levels of beta-endorphin, an index of activation of central pain pathways, was markedly elevated in the placebo group during surgery and at one hour postoperatively in comparison to the local anesthetic group. Pretreatment with a long-acting local anesthetic significantly reduced pain at the 48 hour observation, long after the local anesthetic has dissipated. Subjects also consumed significantly less analgesics over the 24-48 hour time period for pain. These data provide evidence to support the hypothesis that nociceptive afferent barrage produces central plasticity leading to increased postoperative pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00133-21 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assessment of Experimental and Clinical Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gracely, Richard H.	Research Psychologist	NAB NIDR
Others: Dionne, Raymond	Chief, CPU	NAB NIDR
Dubner, Ronald	Chief, NAB	NAB NIDR
Max, Mitchell B.	Chief, CTU	NAB NIDR
Smith, Wendy	Psychologist	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

.77

PROFESSIONAL:

.77

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Four experiments were performed. Two used the track-ball method in which subjects continuously adjust a visual display to assess the intensity of pain sensations evoked by 3-sec thermal stimuli of varying intensity. In the first, 27 subjects rated the intensity of 42 stimuli varying between 43-49°. Both peak response and response duration were associated with stimulus intensity, but the highest association was with the area under the curve which integrates information from both intensity and duration. The second track-ball study presented trains of six 49°C stimuli to assess the effects of the substance P blocker, CP 99,9941, and fentanyl on pain mediated by A delta and C-fiber nociceptors. Fentanyl significantly reduced track-ball ratings of both A delta-mediated and C-fiber-mediated pain sensations evoked at the ankle and arm. Fentanyl had no effect on pain thresholds, and neither CP 99,994 nor placebo altered any measure. The significant efficacy of fentanyl demonstrated the sensitivity of the method, and the negative effect of CP 99,9941 indicates that this substance P blocker had no effect with a method known to produce temporal summation of C-fiber-mediated pain sensation. In a third study, 20 subjects used a simple rating scale and a reaction time button to rate the intensity and latency of pain sensations produced by two thermal stimuli (45 & 47 °C) delivered in a preset protocol both to the same skin location and to varying skin locations. Reaction times to sensations evoked at the same location increased significantly, indicating that the sensation evoked by the first stimuli is mediated by A delta nociceptors and the later responses are to C-fiber mediated heat pain sensations. Repeated stimulation of the same location significantly reduced ratings of sensory intensity ($P < 0.0001$). This simple paradigm provides separate measures of A delta and C-fiber mediated pain sensation useful for clinical evaluation. The fourth study evaluated the influence of pain expectations in the rating and recall of acute pain produced either by venipuncture or dental surgery. Subjects predicted the affective dimension of venipuncture pain but not oral surgery pain. Prediction of the intensity of either pain condition was poor. Affective ratings of venipuncture pain, while predicted accurately, were poorly recalled in comparison to the other ratings. These results suggest that pain recall depends both on the type of pain and the pain dimension that is evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00286-16 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Therapeutics for Acute Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dionne, Raymond A.	Chief, Clin. Pharm. Unit	NAB NIDR
Others: Gordon, Sharon	NRSA Fellow	NAB NIDR
Brahim, Jaime	Oral Surgeon	CIPCB NIDR

COOPERATING UNITS (if any)

Rowan, Janet	Nurse	CC Nursing
Parada, Susan	Nurse	CC Nursing
Maduro, Hilda	Patient Care Technician	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.96

PROFESSIONAL:

0.96

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project consists of a series of clinical trials evaluating the clinical efficacy and safety of experimental therapeutic agents for the control of acute pain and perioperative apprehension in ambulatory patients undergoing minor surgical procedures. The surgical removal of impacted third molars serves as a model of minor surgery with associated intraoperative and postoperative pain and perioperative apprehension. All studies are double-blind with randomly allocated, parallel treatment groups and multiple dependent measures of therapeutic efficacy and clinical safety.

The efficacy of a low dose of a nonsteroidal anti-inflammatory drug (NSAID) ketoprofen applied peripherally at the site of injury was demonstrated in two previous clinical trials. A recent study provides evidence for a locally-mediated mechanism of action: administration into the extraction sites results not only in significantly less pain than following oral administration of the same formulation but with substantially lower blood levels of the drug. The results of these studies support the hypothesis being evaluated that peripheral administration of an NSAID at low doses results in greater analgesic efficacy and a lower incidence of side effects by minimizing systemic exposure. Two parallel studies evaluated the analgesic efficacy of a receptor antagonist of substance P. The drug is administered prior to, during, and following the surgery in an attempt to block occupancy of the substance P receptor and possible CNS activation of nociceptive processes which can persist after removal of the painful peripheral input. Results of the initial study demonstrated a transient effect at one time point in comparison to placebo, with the positive control (ibuprofen) providing a prolonged suppression of pain in the immediate postoperative time point. A subsequent study did not provide any additional evidence to support this observation, suggesting that the analgesic effects of this substance P antagonist cannot be demonstrated in the oral surgery model of acute pain

A study in progress is evaluating the analgesic efficacy of dextromethorphan, an antitussive drug which is also active as a blocker of the N-methyl-D-aspartate (NMDA) receptor. Subjects receive increasing doses of the drug over 48 hours based on side effects reported. One-third of the projected number of patients have successfully completed the study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00288-16 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of the Molecular Response to Noxious Stimulation and Nerve Injury

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ruda, Maryann	Chief, CMM Section	NAB NIDR
Others: Allen, Barbara	Biologist	NAB NIDR
Franklin, Emma	Biological Lab. Tech.	NAB NIDR
Ren, Ke	Visiting Associate	NAB NIDR
Zhang, Rui-Xin	Visiting Scientist	NAB NIDR
Pfaffenroth, Elizabeth	Pre-IRTA Fellow	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.74

PROFESSIONAL:

2.74

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research project extends our analysis of the neuronal response to noxious stimulation of the periphery and nerve injury. Neurons in the dorsal root ganglion and spinal cord represent the first level of processing of neuronal information from the periphery. Using cellular and molecular techniques it is possible to identify important elements in the neuronal networks that subserve the response to nociception, nerve injury and regeneration. The descending control of nociceptive neuronal circuits is being studied in animals with spinal cord transection and inflammation of one hindpaw. Following spinal transection, complete Freund's adjuvant (CFA) hindpaw injection produces edema that is comparable in magnitude to the sham operated control rats. RNA blot analysis of spinal cord dynorphin mRNA regulation identified an 810%, 527% and 500% ipsilateral increase in the spinal transection, sham surgery and naive groups respectively as compared to the contralateral side of the sham surgery group. Immunolabeling of dynorphin immunoreactive cell bodies showed a similar number of neurons in both the spinally transected and sham surgery groups. These data demonstrate that descending afferents to spinal neural circuits partially suppress the dynorphin mRNA and peptide upregulation that occurs in response to peripheral inflammation and hyperalgesia.

The aging process includes a variety of physiological and biochemical changes that might impact neuronal processing of noxious inputs. In CFA treated rats of different age groups, the withdrawal of latency of the aged rats (18 months old) was significantly shorter and the edema was greater than that observed in adult animals. This difference was accompanied by significant increase in spinal dynorphin mRNA levels in aged rats. These data indicate that aged rats exhibit a more robust response to painful stimuli resulting in a greater induction of dynorphin mRNA as a result of peripheral inflammation and hyperalgesia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00329-14 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Discrimination of Thermal Stimuli Applied to the Face in Monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Kenshalo, Jr. Daniel R.	Research Biologist	NAB NIDR
Others: Dubner, Ronald	Chief, NAB	NAB NIDR
Douglass, Diana	Postdoctoral Fellow	NAB NIDR
Sholas, Maurice	Special Volunteer	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, MD 20892

TOTAL STAFF YEARS:

3..03

PROFESSIONAL:

3.03

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the neural mechanisms that subserve the monkey's ability to detect innocuous cutaneous stimuli (air puffs) and noxious stimuli. The magnitude of sensations produced by small increases in air puff stimuli was studied with use of a reaction time paradigm. The monkey initiated a trial by pressing an illuminated button. Subsequently air puff stimuli (AP1) of identical intensity were delivered to the face at the rate of one per second. After a variable time period between 4 and 10 seconds, an air puff of higher intensity (AP2) was presented. The subject was required to release the button as soon as the larger air puff stimuli was detected. Discrimination speed was defined as the reciprocal of the time interval between the onset of the larger air puff stimulus and the release of the button. The discharge of medullary dorsal horn neurons was recorded while the monkey performed the air puff psychophysical task. A subpopulation of wide-dynamic-range (WDR) neurons were found that encode the intensity of innocuous air puff stimuli with an increase in peak discharge frequency. In these neurons, the peak discharge frequency for AP2 is strongly correlated with the animals detection speed. An additional subpopulation of low threshold mechanoreceptive (LTM) neurons was found that encoded the intensity of air puff stimuli and their discharge was highly correlated with the animals detection speed. There was no significant difference between the encoding properties of WDR and LTM neurons. We therefore conclude that subpopulations of both WDR and LTM neurons in the trigeminal nucleus caudalis of the primate can account for the monkey's ability to discriminate innocuous air puff stimulation.

In a second study, we examined the changes in receptive field properties of nociceptive neurons in the primary somatosensory cortex (SI) after an intradermal injection of capsaicin. In humans, an intradermal injection of capsaicin produces burning pain, cutaneous hyperalgesia and allodynia. Neurons that responded maximally to a pinch stimulus received an intradermal injection of capsaicin. After the injection of capsaicin, the discharge rate of all neurons increased dramatically and remained elevated for an average of 75 seconds. The injection also produced an expansion in receptive field size in 22 of 30 neurons. The expansion (10-1500%) was present within 5 min. and persisted for at least 25 min. The responses to a series of graded mechanical stimuli also increased after capsaicin injection with the largest relative increase to the pressure stimulus. These results indicate that nociceptive neurons in primate SI cortex respond to intradermal injection of capsaicin and that tonic activity in nociceptive pathways can alter receptive field size and responses to mechanical stimulation. We conclude that many of the receptive field changes seen in nociceptive SI neurons can account for the burning pain, cutaneous hyperalgesia and allodynia in humans after injection of capsaicin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00366-13 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analgesic Mechanisms in Patients with Chronic Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Max, Mitchell B.	Chief, Clinical Trials Unit	NAB NIDR
Others: Sang, Christine N.-M.	Clinical Associate	NAB NIDR
Nelson, Kristine	IRTA Fellow	NAB NIDR
Liu, Maywin	Special Volunteer	NAB NIDR
Gracely, Richard H.	Research Psychologist	NAB NIDR
Bennett, Gary J.	Chief, NPPM Section	NAB NIDR

COOPERATING UNITS (if any)

Robinovitz, Elaine	Nurse	CC Nursing
Parada, Sue	Nurse	CC Nursing

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain and Pain Measurement Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.70

PROFESSIONAL:

3.70

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to elucidate the neural mechanisms and principles of treatment of chronic pain syndromes, with particular attention to the drug treatment of pain caused by nerve injury. Because of growing evidence from animal studies that neuropathic pain may be mediated by N-methyl-D-aspartate (NMDA) receptor-mediated activation of spinal cord neurons, a focus of recent work has been the evaluation of NMDA receptor antagonists in patients with neuropathic pain. Twenty-six patients (13 with painful diabetic neuropathy and 13 with postherpetic neuralgia) completed a placebo-controlled double-blind crossover trial of the NMDA receptor antagonist dextromethorphan, 400mg/day (approximately 3 1/2 times the maximum dose recommended for dextromethorphan as a cough suppressant). In the diabetic patients, pain was reduced by a mean of 24% relative to placebo ($p = 0.014$); 7/13 patients reported moderate or greater relief with dextromethorphan, compared to 0/13 with placebo. Pain was minimally affected by dextromethorphan in the patients with postherpetic neuralgia, however. Drug side effects, including sedation, confusion, and dysphoria, caused four patients to drop out, but an acceptable dose regimen could be found for the other patients. This is the first study showing that chronic treatment with an NMDA receptor antagonist relieves pain and is reasonably well tolerated. This result supports the hypothesis, generated in Bennett's CCI neuropathic rat model, that NMDA receptor mechanisms are important in the generation of neuropathic pain. If confirmed by additional studies, this clinical trial makes a new treatment available for patients with neuropathic pain syndromes, including peripheral neuropathy in diabetes, AIDS, and other systemic diseases, and orofacial syndromes such as traumatic nerve injury, reflex sympathetic dystrophy, and atypical facial pain. Current studies in patients with neuropathic pain are comparing dextromethorphan to memantine, another NMDA receptor antagonist, and placebo, with the intent of confirming the efficacy of dextromethorphan, and finding a more effective and safer alternative. Other work this year included four studies of the drug effects and sensory mechanisms in the intradermal capsaicin model in normal volunteers, which transiently reproduces symptoms resembling neuropathic pain, and collaborative studies with other centers of acupuncture, mexiletine, and amitriptyline in pain caused by AIDS-related neuropathy, and of morphine in post-herpetic neuralgia. Our work in developing methods for doing clinical studies of chronic pain has resulted in 10 publications this year on analgesic clinical trial methodology for patients with pain caused by neurological disease, surgery, arthritis, cancer, and sickle cell disease, in draft guidelines for the FDA, and in a course on clinical trials at the upcoming Eighth World Congress on Pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00413-10 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Experimental Neuropathy of Peripheral Nerve in Rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Bennett, Gary J.	Research Biologist	NAB
Others: Xiao, Wen-Hua	Visiting Fellow	NAB NIDR
Imamura, Yoshiki	Special Volunteer	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain and Pain Measurement Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.77

PROFESSIONAL:

1.77

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

People whose peripheral nerves have been damaged by trauma or disease sometimes develop chronic pain syndromes that are difficult or impossible to treat. This project investigates this problem with the aide of an experimentally-induced painful peripheral neuropathy in rats that is created by tying loosely constrictive ligatures around the sciatic nerve (the CCI model) or around a branch of the trigeminal nerve, the infraorbital nerve (the PTN model). The animals have abnormal pain sensations like those seen in humans. In particular, they have hyperalgesia (exaggerated responses to painful stimulation) to thermal and mechanical stimuli, allodynia (pain from normally innocuous stimuli) to touch, and spontaneous pain (or dysesthesia). A recently introduced anti-epileptic drug, felbamate, was found to suppress hyperalgesia and allodynia in the CCI model, without having any effect on the pain responses of normal rats. The anti-hyperalgesic and anti-allodynic effects were obtained with systemic doses that did not produce side-effects. Recent clinical reports indicate that felbamate has an unacceptable safety profile. Nevertheless these results suggest that a drug cocktail mimicking felbamate's mechanisms of action may be useful. Another recently introduced anti-epileptic, gabapentin, was shown in the CCI model to suppress heat-hyperalgesia and mechano-allodynia, but not mechano-hyperalgesia, at doses that did not produce side-effects. Comparable effects were obtained when the drug was administered intrathecally, suggesting that the mechanism of action is at the level of the spinal cord. Interruption of the efferent sympathetic innervation of the painful body region is known to eliminate or reduce neuropathic pain in some patients. Previous studies, however, have shown that sympathectomy has little or no effect in CCI rats. In contrast, we have recently shown that sympathectomy (stellate ganglionectomy) blocks heat-hyperalgesia in PTN rats. These results indicate that comparable injuries to different nerves may produce pain syndromes with fundamentally different underlying mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00414-10 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

CNS Neurotransmitter Regulation During Peripheral Inflammatory States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Iadarola, Michael J.	Research Pharmacologist	NAB NIDR
Others:	Gu, Jun	Visiting Fellow	NAB NIDR
	Messersmith, Donna J.	IRTA Fellow	NAB NIDR
	Mannes, Andrew J.	Guest Researcher	NAB NIDR
	Dubner, Ronald	Chief, NAB	NAB NIDR
	Wu, Pei-Yun	HHMI Summer Student	NAB NIDR
	Getek, Kathryn	HHMI Summer Student	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.06

PROFESSIONAL:

3.86

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focuses on the transcriptional control of the prodynorphin gene, which codes for the dynorphin family of opioid peptides. Peripheral inflammation greatly increases dynorphin gene expression in spinal cord neurons where peptides can modulate chronic pain. Transient transfection using in vitro cell lines indicate that the dynorphin gene is turned on by the cyclic AMP (cAMP) second messenger system. A dual feed-forward stimulatory transcription scheme is proposed in which members of the CREB/ATF and Fos/Jun families positively transactivate the dynorphin gene. The results imply that a transmitter in primary afferent neurons is linked to stimulation of adenylyl cyclase in second order neurons and that the gene regulatory and neuronal excitability changes which accompany chronic pain may be engendered or maintained by cAMP-dependent phosphorylation. The cAMP responsive element is located at -1546 bp from the transcription start site. Constructs focused solely on this element using a 41 bp oligonucleotide displayed a 40 to 150-fold enhancement of chloramphenicol acetyltransferase reporter gene expression when cells were stimulated with forskolin. The forskolin-induced increase could be attenuated by expression of a transactivation mutant of the c-Jun protooncogene indicating the participation of Jun in endogenous pathways of dynorphin gene expression. Additional functional assessments of the DYNCRE3 sequence were performed with a series of DYNCRE3 mutations. The data demonstrate that (a) an intact core heptanucleotide region is required for optimal basal and inducible expression, (b) the immediate two flanking bases are of minimal importance, (c) nucleotides further from the core influence activity but less so than core mutations, and (d) under different conditions of stimulation the DYNCRE3 element exhibits characteristics of an AP-1 element or a CRE element. These data indicate that the DYNCRE3 element provides a more flexible (and complex) transcriptional responsiveness than either of the two consensus sequences. Based on the above in vitro observations we have begun corresponding experiments using the rat peripheral inflammation model. These include examination of Jun and CREB proteins and their phosphorylation status and assessment of the effects of modulation of the cAMP system on nociceptive sensitivity and neuropeptide gene. The significance of our studies to biomedical research is found at the molecular and neural circuit levels and in the new directions for pain control they provide. Spinal cord neurons undergo a pronounced, sustained activation of an entire gene regulatory cascade in response to persistent nociceptive input, as occurs with inflammation, traumatic pain, pain associated with arthritis and possibly with cancer. We have obtained fundamental new insight into the processes controlling transcription of the dynorphin gene, which codes for a family of endogenous opioid peptides, and the second messenger pathways involved. Further elucidation of the pivotal role of the spinal dynorphin system may provide new avenues for the pharmacotherapy of pain and insights into chronic opioid use and tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00440-09 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Descending Modulation And Dorsal Horn Plasticity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ren, Ke	Visiting Associate	NAB
Others: Dubner, Ronald	Chief, NAB	NAB NIDR
Ruda, Maryann	Chief, CMM Section	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, MD 20892

TOTAL STAFF YEARS:

.834

PROFESSIONAL:

.834

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This study was undertaken to examine the neural mechanisms underlying persistent inflammation and hyperalgesia. The experiments were designed to study the modulation of dorsal horn hyperexcitability and behavioral hyperalgesia by brain centrifugal systems. The dorsolateral funiculus (DLF) of the spinal cord contains descending pathways that are important for nociceptive modulation at the spinal level. To reveal the role of descending mechanisms in the development of central sensitization and hyperalgesia in response to persistent peripheral neural barrage, the effects of bilateral lesions of the dorsolateral quadrant of the spinal cord (DLFX) on inflammatory hyperalgesia were studied. Inflammation was induced by injection of complete Freund's adjuvant (CFA) or carrageenan (CARRAG) into one rat hindpaw and paw withdrawal (PW) response to a thermal stimulus was tested. Following the injection of CFA, a similar magnitude of thermal hyperalgesia developed in the injected paws of sham-operated and non-operated naive (SH, n=8), and DLFX (n=7) rats. However, when the thermal stimulus was adjusted to a lesser intensity and a low dose of CARRAG (1 mg) was injected, a significantly stronger hyperalgesia was exhibited in DLFX rats (n=6), when compared to SH rats (n=8) (ANOVA, $F_{1,14}=14.04$, $p<0.01$). Furthermore, a contralateral hyperalgesia that is not typically seen in SH rats was unmasked within 0.5-2 h after the injection of CARRAG in DLFX rats. Compared to both groups of SH rats, the potency of morphine in inhibiting the PW response was reduced in non-inflamed DLFX rats, and further reduced in inflamed DLFX rats. The relative potencies of morphine in different groups of rats were: inflamed SH (1.00) > SH (0.62) > non-inflamed DLFX (0.16) > inflamed DLFX (0.09). These results suggest that the development of inflammatory hyperalgesia is counteracted by descending inhibitory mechanisms. Both descending inhibitory mechanisms and the suppression of spinal hyperexcitability contribute to the anti-hyperalgesic potency of morphine after inflammation. We further compared the involvement of specific brain stem sites, nucleus raphe magnus (NRM) and lateral reticular nucleus (LRN), in the modulation of spinal nociceptive neurons in CFA-inflamed rats. A local anesthetic block was produced by microinjection of lidocaine into the NRM or LRN and the changes in nociceptive neuronal activity were examined. Following the NRM lidocaine block, an increase in neuronal activity was observed in the majority of neurons, although occasionally reduced responsiveness was also seen. In contrast to the NRM lidocaine, a smaller population of dorsal horn nociceptive neurons showed an increase in activity following the LRN lidocaine block. These results suggest that the NRM and LRN may be differentially involved in plastic changes in descending pathways in the event of persistent peripheral inflammation. These studies characterize physiological and pharmacological mechanisms that contribute to the development of spinal hyperexcitability and behavioral hyperalgesia in animal models of peripheral tissue injury. A better understanding of these mechanisms may lead to improvement of the treatment of chronic pain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00509-06 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Neuropathic Pain in Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Gracely, Richard	Research Psychologist	NAB NIDR
Others: Bennett, Gary	Chief, NPPMS	NAB NIDR
Dubner, Ronald	Chief, NAB	NAB NIDR
Max, Mitchell	Chief, CPU	NAB NIDR
Smith, Wendy	Psychologist	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Neuropathic Pain and Pain Measurement Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.77

PROFESSIONAL:

0.77

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project uses both patients and normal volunteers to investigate the activation and maintenance of mechanisms responsible for central enhancement of spontaneous and evoked pain. Two patients with persistent localized inflammatory disease were examined and particularly painful foci (4 to 5) were each injected with 0.1 to 0.2 ml of 2 % lidocaine. During the 20 min of anesthesia, spontaneous pain was reduced or abolished, and touch applied to previously painful, unanesthetized areas was perceived as touch and not as pain. This result and similar results in 4 patients last year further confirms our model of altered central processing maintained by input from nociceptors and supports the concept that the variety of syndromes may reflect the various means by which this input may be achieved. Previous findings in patients suggest that focal input from a specific nerve territory can result in allodynia and spontaneous pain in the territory of afferent peripheral nerve, a finding that has often resulted in a psychiatric diagnosis. A series of radial and ulnar nerve blocks defined the extent of these nerve territories in the dorsum of the hand in 12 subjects. Intradermal injection of capsaicin into the ulnar distribution resulted in allodynia and secondary hyperalgesia in the radial nerve distribution, confirming clinical findings and consistent with our hypothesis that allodynia and other effects result from a central mechanism. Three studies of 10 subjects each showed that electrically-evoked sensations are reliable even when the electrodes are removed and reapplied, validating this important technique. An intradermal capsaicin injection facilitated temporal summation in 10 subjects, implicating summation mechanism in clinical conditions of central hyperexcitability. A study (n=10) of the influence of innocuous A beta stimulation was inhibitory when delivered before capsaicin induced facilitation of the nociceptive reflex found that A beta stimulation was inhibitory when delivered before capsaicin intradermal injection and excitatory when delivered after the injection. These effects represent both classic "Gate Control" pain inhibition and the novel finding that once initiated, altered central processing can be maintained by input from touch fibers. New studies will examine the duration of pain sensations produced by heat applied to the zone of allodynia, the effect of trains of thermal and electrical stimulation applied to this zone, and use a nociceptive reflex to measure A beta maintenance of central summation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00532-05 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathophysiology of Chronic Orofacial Pain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: DeNucci, Donald	Visiting Scientist	NAB NIDR
Others: Dionne, Raymond	Chief, CTU	NAB NIDR
Dubner, Ronald	Visiting Scientist	NAB NIDR
Brahim, Jamie	Oral surgeon	CIPCB NIDR
Reid, Kevin	Visiting Scientist	NAB NIDR
Meehan, Sean	Oral Medicine Fellow	NAB NIDR

COOPERATING UNITS (if any)

Chen, Clara	Staff Physician	NM CC
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LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.44

PROFESSIONAL:

1.44

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is attempting to better characterize the pathophysiology of chronic orofacial pain through a series of clinical investigations. A clinical study in progress is evaluating the relationship between sleep disorders and temporomandibular disorders (TMD) which are thought to result in nocturnal muscle hyperactivity leading to pain in the muscles of mastication especially upon awakening. A double-blind crossover study is being conducted in which subjects receive either triazolam, a benzodiazepine hypnotic, or placebo over the course of five days with concurrent monitoring of pain and sleep architecture. Following a washout period of three days, subjects receive the alternative treatment and are monitored similarly. Documentaion of an improvement in the quality and quantity of sleep by polysomnography and a parallel change in pain in the temporomandibular region is interpreted as evidence of a relationship between sleep disorders and orofacial pain. Interim data analysis suggests a functional relationship between alterations in sleep architecture, sleep quality, and pain in the muscles of mastication. A second study is investigating arthrocentesis of the temporomandibular joint (TMJ) as a possible therapeutic modality and as a means to collect fluid and cells from the TMJ for later analyses. This study uses a randomized double-blind design to administer either a saline wash or an anesthetic agent to the TMJ and then to assess the effect on pain and mandibular range of motion. The arthrocentesis is performed with a novel coaxial needle technique developed by the primary investigator. Preliminary findings suggest a therapeutic benefit to a saline lavage of the TMJ and demonstrate the efficacy of the coaxial needle device in performing arthrocentesis in TMD patients. A third study involves an investigation of an auriculotemporal nerve block using either a local anesthetic or saline as a possible diagnostic procedure to assist in the determination of the source of facial pain in TMD patients. Using a randomized double-blind design, thirty-five patients with TMJ pain received an auriculotemporal nerve block with either lidocaine 2% or saline. Pain and range of motion measurements were collected both before and after the block. An interim analysis of data supports the use of an auriculotemporal nerve block as a diagnostic procedure in TMD patients and suggests that some of muscle pain perceived by these patients may be augmented by input from the TMJ. A fourth study involves an investigation of idiopathic jaw pain using single photon emission computed tomography (SPECT) to determine whether or not this pain might be the result of a neuralgia induced subacute osteomyelitis. Patients are clinically evaluated to eliminate any organic etiologic factors responsible for their pain. SPECT images of the head and neck are then obtained and subsequently evaluated by a nuclear medicine physician who is unaware of the location of patient's pain. The relationship between regions of radio-isotope uptake with the area of pain within the jaws is being evaluated. Preliminary findings in sixteen patients with idiopathic jaw pain lend tentative support to the hypothesis that idiopathic jaw pain is the result of a localized subacute osteomyelitis in some patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 DE 00556-04 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptide Interactions with Excitatory Synapses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Caudle, Robert M. Staff Fellow NAB NIDR
Others: Dubner, Ronald Chief, NAB NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIH, NIDR, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.067

PROFESSIONAL:

1.067

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project focused on the regulation of the N-methyl-D-aspartate (NMDA) subclass of excitatory amino acid receptors by the endogenous opioid peptide dynorphin. We demonstrated in the guinea pig hippocampal slice preparation that dynorphin can act as an agonist at two distinct receptors to regulate the function of NMDA receptors. The first site that dynorphin acts at in the hippocampal slice are the kappa2 opioid receptors. Dynorphin binding to this site results in an inhibition of the current that flows through the NMDA receptors. Prior to this discovery there was no known physiological function for the kappa2 opioid receptor. The second site that dynorphin acts at is the polyamine binding site located on the NMDA receptor. When dynorphin binds to this site the current flowing through the NMDA receptor is enhanced. The enhancement of the NMDA receptor-mediated current by dynorphin often results in neuronal damage and death in vitro. This finding is similar to the effect observed when dynorphin is injected onto the spinal cord of rats in vivo, suggesting that dynorphin's neurotoxic effects in vivo are mediated through the polyamine site of the NMDA receptor. It is well established that excessive activity in NMDA receptors may result in many different neuropathologies, including chronic pain. It is also well established that many of these pathologies are associated with elevated levels of endogenous dynorphin. Therefore, it is possible that endogenous dynorphin may play a role in enhancing the activity of NMDA receptors during pain. We are testing our hypothesis in a behavioral model of persistent pain. In rats whose right hind paw is inflamed by the injection of complete Freund's adjuvant, opioid agonists which act at the kappa2 opioid receptor inhibit the inflammation induced hyperalgesia when injected onto the spinal cord. The sensory profile of the non-inflamed paws are not altered by the kappa2 agonists. This finding is in contrast with the effects of other opioid agonists which alter the sensory profile of both the inflamed and the non-inflamed paws. The findings do, however, resemble results obtained by the injection of NMDA receptor antagonists, suggesting that the kappa2 receptors in vivo are regulating the NMDA receptors. Future work will focus on determining the mechanism by which kappa2 opioid receptors inhibit NMDA receptors and on the further characterization of the kappa2 opioid receptors and the polyamine binding site in the behavioral model.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 DE 00599-03 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Somatosensory Studies of Pain and Pain Control Measured with PET and Functional MRI

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Iadarola, Michael	Research Pharmacologist	NAB NIDR
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Coghill, Robert	IRTA Fellow	NAB NIDR
Dionne, Raymond A.	Chief, Clin. Pharm. Unit	NAB NIDR
Gracely, Richard H.	Research Psychologist	NA B NIDR
Kenshalo, Jr., Daniel R.	Research Biologist	NA B NIDR
Max, Mitchell B.	Chief, Clinical Trials Unit	NAB NIDR

COOPERATING UNITS (if any)

Berman, Karen F.	Chief, PET Imaging Unit	CBDB NIMH
Balaban, Robert S.	Chief, Lab, Cardiac Energetics	LCE NHLBI
Wen, Han	Guest Resercher	LCE NHLBI

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Nociception and Tissue Injury Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.65

PROFESSIONAL:

1.65

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this clinical research effort is to understand how the human central nervous system processes pain information and to identify abnormalities in CNS pain processing in patients with neuropathic and/or chronic pain conditions. High resolution (a) positron emission tomography (PET) with radioactive tracers or (b) functional magnetic resonance imaging (fMRI) techniques are used to assess regional brain activity via blood flow changes. These methods allow us to employ state of the art functional and structural brain imaging techniques to the study of normal and pathological pain states in human beings. Over the past year we have established our data analysis workstation and fully implemented several basic brain imaging analysis methodologies for PET data sets, and systems for data archiving. Oxygen-15 water blood flow PET studies were conducted on 15 normal volunteers and 14 patients with unilateral post-herpetic neuralgia. The major normal volunteer study involved a fully quantitative evaluation of regional cerebral blood flow during acute capsaicin-induced pain. We discovered that capsaicin induced a massive decrease in global blood flow. This decrease (30%) could not be accounted for by changes in arterial pCO₂, heart rate or respiratory rate. This pain induced decrease is a new and previously unreported alteration in cerebral blood flow induced by a physiological stimulus. It is rapid in onset, large in magnitude, transient in duration, and may represent a novel cerebrovascular regulatory mechanism evoked by strong acute pain. Our PET studies of chronic pain associated with post-herpetic neuralgia (PHN) suggest that pain induced from the neuropathic zone by a light tactile stimulus induces activity in a network of brain regions containing both nociceptive and non-nociceptive somatosensory components. These patients are elderly and control PET data from a group of age-matched normal subjects was obtained via collaboration with the Laboratory of Neurosciences, NIA. The multislice fMRI studies investigated the influence of increasing levels of activation on the spatial distribution of the fMRI signals. The data indicate that increasing levels of movement or noxious thermal stimulation result in increased signal intensity that retains its spatially discrete nature. Our data suggest that we can discriminate large versus small caliber vessels based on MR signal magnitude, although several alternative explanations need to be explored as well. MRI provides a powerful new tool for investigation of dynamic aspects of human pain and its disorders.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 DE 00614-02 NAB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Genes Regulated During Neuronal Injury And Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: MacArthur, Linda	Staff Fellow	NAB NIDR
Others: Ruda, Maryann	Chief, CMMS	NAB NIDR
Allen, Barbara	Biologist	NAB NIDR
Franklin, Emma	Biological Lab Tech	NAB NIDR

COOPERATING UNITS (if any)

LAB/BRANCH

Neurobiology and Anesthesiology Branch

SECTION

Cellular and Molecular Mechanisms Section

INSTITUTE AND LOCATION

NIDR, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.15

PROFESSIONAL:

2.15

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular mechanism by which brief periods of neuronal activity leads to long-lasting changes in the nervous system is thought to involve activation of novel patterns of gene expression, which results in alteration of the physiology of the cell. To understand the mechanisms by which chronic pain and nerve injury alter the nervous system, as well as to identify putative biological regulators involved in these conditions, the molecular mechanisms behind pain and nerve injury need to be better understood.

The best characterized genes which are regulated by the pain and nerve injury pathways in the nervous system are the neuropeptide genes. These genes contain unique combinations of cis-acting DNA elements through which signaling has been demonstrated to occur in vitro. Furthermore, individual neuropeptides demonstrate differential responsiveness to the same stimulus making them ideal endogenous "reporter genes" for studying signal transduction of specific stimuli in the nervous system.

Using standard gel shift methods, we have demonstrated that spinal cord proteins that bind to the enkephalin enhancer sequences are different from those that bind either chromaffin cells or PC12 cells. Furthermore, this binding activity is specific for the enkephalin enhancer since it can differentiate between enhancers with a similar core sequence but different flanking sequences. Therefore, transcription factors which bind to the enkephalin gene, and likely other neuropeptide genes, are both tissue specific and highly selective for target DNA elements within a tissue. Using neuropeptide genes at the level of the spinal cord and dorsal root ganglia that are responsive to nerve injury and pain, we will focus on identifying transcription factors which are specific for these signaling pathways in vivo.



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